EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF PSYCHOPHARMACOLOGIC DRUGS ON EXPERIMENTAL SEIZURE THRESHOLD

by

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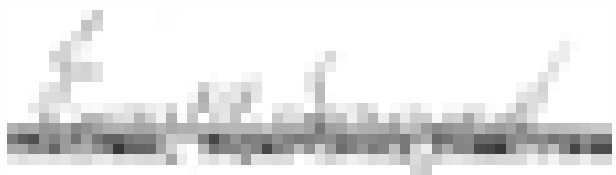

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TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd.)

LIST OF TABLES

LIST OF FIGURES

I. GENERAL INTRODUCTION

Since drug addiction touches upon many areas of human endeavor (sociology, law, medicine, etc.), it has been difficult to develop a definition for the term which is acceptable to everyone concerned. The most generally accepted definition was formulated in 1950 by the Expert Committee on Drugs Liable to Produce Addiction of the World Health Organization. This committee defined the term as follows (1950): "Drug addiction is a state of periodic or chronic intoxication, detrimental to the individual and to society, produced by the repeated consumption of a drug (natural or synthetic). Its characteristics include: (1) an overpowering desire or need (compulsion) to continue taking the drug and to obtain it by any means; (2) a tendency to increase the dose; (3) a psychic (psychological) and sometimes a physical dependence on the effects of the drug." To clarify the distinction between addiction and habituation (habit), this committee subsequently reported (1952) that habituating drugs never produce compulsive craving, can be interrupted after chronic administration without significant physical or psychological disturbances, and cause no sociological damage.

Two terms used in the above definition, psychic and physical dependence, require further clarification. Psychic dependence or emotional dependence is hard to define, but, according to Wikler (1953), means simply that the addict feels discontented or behaves as if he feels discontented, or anxious or unhappy unless he is under the influence of a drug. On the other hand, physical depend-

ence refers to the development of an altered physiologic state, consequent to repeated use of a drug over a period of time, which requires continued administration of the drug to prevent the appearance of a characteristic illness, termed an abstinent syndrome.

Although the term tolerance is not specifically mentioned in the definition, it is implied by the words "tendency to increase the dose." Tolerance refers to the progressive diminution in the intensity of the pharmacological effects evoked by the same dose of a drug during continuous and regular use (Wikler, 1953). According to Tatum and co-workers (1929), tolerance is an altered physiological state induced by the chronic administration of certain drugs such that progressively larger and larger quantities are required to produce the effects desired. Thus, it would appear that the development of tolerance is an important component of drug addiction, according to the definition cited above.

Several additional aspects of the definition for addiction should be mentioned. Addiction usually involves abuse or improper use of drugs. Also, it should be noted that although physical dependence is generally a characteristic of addiction, according to the definition of the World Health Organization, it is not a necessary feature. Isbell and Fraser (1950) emphasized that any definition which makes physical dependence an essential feature of drug addiction will exclude cocaine, marihuana, and certain sympathomimetic amines (e.g. amphetamine) as addicting drugs, since

little or no physical dependence develops to these agents. The word "periodic" is included in the first sentence of the definition for addiction, because cocaine and marihuana are used by drug addicts as "spree" drugs and are not taken continuously.

A. The Etiology of Addiction

Despite the numerous studies on the subject, the etiology of drug addiction is not clearly understood. Psychiatric and psychologic studies on drug addicts (Pescor, 1938, 1941; Reichard, 1943, 1947; Vogel et al., 1948; Wikler 1953) indicate that a personality defect is the most important factor which predisposes an individual to addiction. The types of personalities which usually underlie drug addiction were originally classified by Kolb (1925) and Kolb and Ossenfort (1938) into 6 groups and subsequently limited by Felix (1944) to 4 general classes. The first class, which comprises a very small percentage of addicts, consists of normal individuals accidentally addicted as the result of legitimate therapeutic use of a drug. These individuals are often referred to as medical addicts. Although these individuals may not appear to have personality defects, Vogel and co-workers (1948) are of the opinion that they do have fundamental emotional problems which cause them to continue the use of drugs beyond the period of medical need. Hence, according to these authors, there is no basic difference between these so-called medical addicts and the non-medical addicts. The second class consists of psychoneurotics who take drugs to relieve various symptoms, whether somatic or psychic.

The third class consists of psychopathic inferiors who are generally emotionally undeveloped aggressive hostile persons. These individuals take drugs merely for the pleasure arising from the unconscious relief of inner tension. The fourth and smallest class consists of addicts with associated minor psychoses. Major psychoses apparently play no role in the genesis of drug addiction (Isbell, 1950a). In general, a vast majority of drug addicts have difficulty in making a satisfactory adjustment to life. Their adaptive patterns of behavior are inadequate and they frequently find in drugs a means of escape from the stresses and problems of life.

B. Addicting Drugs

On the basis of the definition for addiction as set forth by the World Health Organization, Isbell and White (1953) consider the following agents as the important addicting drugs in the United States: (1) opiates and synthetic analgesics (opium, laudanum, paregoric, morphine and morphine derivatives, methadone, and meperidine); (2) hypnotic and sedative drugs (barbiturates, chloral hydrate, paraldehyde, and bromides); (3) alcohol; (4) cocaine; (5) certain sympathomimetic amines (amphetamine and methamphetamine); (6) mescaline (peyote) and (7) marihuana.

C. Symptoms of Addiction

Since the various drugs listed above differ in symptoms of intoxication and abstinence, it is of interest to describe the

general effects of these agents. Addiction to morphine may be considered as a prototype of addiction to narcotic-analgesic drugs. The symptoms of opiate addiction have been described by numerous authors (Wikler, 1953; Goodman and Gilman, 1955; Isbell, 1950a, 1950b; Isbell and Fraser, 1950; Himmelsbach, 1941; Isbell and White, 1953). Addiction to opiates is characterized by 3 closely interrelated phenomena: psychic dependence, physical dependence, and tolerance. When an addict first begins the use of opiates he experiences a pleasant relaxation and develops a peculiar semisomnolent state with brief alternating periods of light sleep and wakefulness. If the drug is administered intravenously, a pleasurable tingling spreads over the body. This sensation has been compared to a sexual orgasm, localized in the abdominal region (Wikler, 1952). As the addict becomes tolerant, his temperature and blood pressure which were previously reduced by the drug and his respiratory and pulse rates which were previously lowered return to normal. His semisomnolent state disappears and, if he obtains an adequate supply of drug, his overt behavior appears normal and he can carry on his occupation or profession satisfactorily. The only signs of addiction which may be observed in such an individual are needle marks, constricted pupils, and decreased libido. When morphine is abruptly withdrawn a series of symptoms appear which reach a peak intensity approximately 48 hours after the last dose of drug. The character and severity of the abstinence syndrome depend mainly on the intensity

of physical dependence, which is in turn associated with factors such as total daily dose used, the frequency of drug administration, and the health and personality of the addict. Symptoms of morphine abstinence include lacrimation, rhinorrhea, perspiration, yawning, restlessness, nervousness, gooseflesh, mydriasis, mild hypertension, hyperpnea, fever, leukocytosis, and hyperglycemia. The addict becomes very uncomfortable; he experiences muscle cramps, muscle tremors or twitching, nausea, and vomiting. He experiences diarrhea and loses from 5 to 15 pounds of weight in 24 hours. Approximately 1 week after the final dose of morphine, most of the symptoms of withdrawal disappear. About 6 months is required for physical recovery, as indicated mainly by recovery of original body weight. Abstinence from the other narcotic analgesics differs from morphine abstinence chiefly in the time course and intensity (Isbell, 1950a).

In general, the effects of chronic intoxication and abstinence produced by the various central nervous system depressant agents are very similar (Seevers, 1958; Isbell and White, 1953); therefore, barbiturate addiction can be discussed as the prototype of addiction to this class of agents, including ethyl alcohol. There is a large number of experimental and clinical reports to indicate that if large doses of barbiturates are taken chronically, tolerance and physical dependence develop and a characteristic withdrawal syndrome appears when the drug is suddenly stopped or the dosage markedly reduced. (Dunning, 1940; Kalinowsky, 1942;

Isbell, Altschul et al., 1950; Fraser and Isbell, 1954; Fraser et al., 1954; Isbell and White 1953; Isbell, 1950a, 1950b, 1951). Although the long-acting barbiturates are sometimes used by addicts, the potent short-acting agents, pentobarbital, secobarbital, and amobarbital are more popular. Chronic intoxication with barbiturates results in impaired mental function, loss of emotional control, poor judgment, confusion, and, occasionally, a toxic psychosis. Also, nystagmus, dysarthria, ataxia, and adiadochokinesia are prominent signs. Individuals who are addicted only to barbiturates continue to eat and to maintain a good state of nutrition. The respiratory rate and minute volume are not greatly depressed and coma is uncommon. Partial tolerance to the barbiturates develops, but it is never complete; thus, tolerance to the lethal effect of barbiturates does not develop. Upon abrupt cessation of the barbiturate, intoxication initially declines and the addict appears to improve. Then he experiences anxiety, nervousness, tremor, weakness, impaired cardiovascular responses, and muscle tremors. Approximately 2 days after withdrawal from a short-acting barbiturate or about 1 week after withdrawal from a long-acting agent many addicts experience grand mal type convulsions. Other addicts may experience insomnia which culminates in delirium. Agitation may lead to extreme exhaustion and death (Fraser et al, 1953). Chronic ethanol intoxication differs somewhat from barbiturate intoxication in that there are usually nutritional dis-

turbances associated with the former situation. Also, cirrhosis of the liver frequently occurs in chronic alcoholic persons. Addiction to other aliphatic hypnotics such as chloral hydrate and paraldehyde is not a problem in the United States (Isbell and White, 1953; Seevers, 1958). According to the review of Pohlisch as cited by Kalinowsky (1942) and Dunning (1940), the symptomology of intoxication and withdrawal of chloral hydrate and paraldehyde resembles that of alcohol and the barbiturates. Bromide addicts show psychic dependence, but apparently do not develop tolerance or physical dependence (Vogel et al., 1948; Isbell and White, 1953).

Cocaine is capable of causing psychic dependence (Vogel et al., 1948), but does not induce physical dependence or tolerance (Vogel et al., 1948; Seevers, 1958; Tatum and Seevers, 1931). When the drug is taken intravenously, it produces an orgastic sensation which prompts the addict to repeat the injection of cocaine at very short intervals in order to experience this ecstasy as often as possible. As the dose is repeated the toxic effects of cocaine accumulate and a characteristic psychosis develops. The toxic effects of cocaine are so unpleasant that there are few pure cocaine addicts in the United States.

The symptomology of intoxication to the sympathomimetic amines resembles that of intoxication to cocaine (Isbell and White, 1953; Freyhan, 1949). Tolerance develops rapidly to the drug and, except for weakness and depression (Monroe and Drell, 1947) and somnolence (Knapp, 1952), there is no pronounced symptoms of abstinence.

Marihuana is used mainly as a "spree" drug. In a chronic experiment in human subjects, Williams and co-workers (1946) showed that the principal effects of marihuana were initial exhilaration and euphoria followed in a few days by general lassitude and indifference. In isolated instances, marihuana may produce bizarre effects of an antisocial nature. A small degree of tolerance was demonstrated by the fact that the initial loquaciousness and gaiety, which lasted only a few days, could be recaptured by increasing the dose of marihuana. However, no physical dependence was demonstrated.

The use of peyote is almost exclusively associated with religious activities of certain Indian tribes in the United States and Mexico. Intoxication with peyote or mescaline resembles that with marihuana (Walton, 1938). The ingestion of peyote results in visual and auditory hallucinations (Hoch, 1952). As far as is known, tolerance does not develop to mescaline and there are no withdrawal symptoms (Isbell and White, 1953).

D. Experimental Addiction Studies

In the study of addiction liability of various agents, humans as well as infrahuman species have been employed. The literature on experimental morphine addiction has been extensively reviewed by Eddy (1941). The principal components which may be investigated experimentally are psychic dependence, physical dependence, and acquired tolerance. Seevers (1948) and Isbell (1948) have emphasized that psychic dependence must be determined in man, and

pointed out that clinically applicable information from animals is limited to studies of tolerance and physical dependence. The nature of the abstinence syndrome observed depends upon the physical characteristics of the species and the phylogenetic development of the central nervous system (Seevers, 1948). In the lower species, generalized hyperirritability is the only constant sign of withdrawal.

In the use of human subjects for addiction liability studies, 2 general approaches are available for the investigation of narcotic analgesics. The first approach involves the use of patients with intractable pain who require relief for long periods (Isbell and Fraser, 1950). Low doses of analgesics are given to these subjects and a low grade of dependence is produced. The results obtained from this type of study are difficult to interpret and the conclusions derived therefrom are unreliable. For example, such analgesics as heroin, dilaudid, meperidine, and methadone, have been judged to be non-addicting by this technic. The second approach involves the use of Federal prisoners who are active- or post-morphine addicts; this type of study is performed mainly at the Public Health Service Hospital, Lexington, Kentucky. The 4 procedures employed at Lexington have been reviewed by Isbell (1948). The first of these technics is related to the production of euphoria by a single dose of the analgesic; such signs as increased talkativeness, boasting, increased motor activity, etc. are considered as indicative of euphoria. The

second technic consists of determining the ability of the test agent to relieve abstinence from morphine in addicted subjects. The hourly point score system of Himmelsbach (1937) is employed to quantitate the intensity of the withdrawal symptoms; if the test agent has the ability to reduce the severity of or abolish morphine abstinence, it is fairly likely that it is capable of producing physical dependence. The third method involves the abrupt substitution of the new drug for morphine in addicts. If the test drug is capable of supporting the addict and if withdrawal symptoms appear upon abrupt termination of drug administration, it is regarded as having physical dependence liability. The fourth method involves the chronic administration of the test agent to post-addicts in an attempt to induce direct addiction. The drug is administered at the appropriate intervals each day and the dosage is increased from time to time according to the subject's tolerance to the toxic effect of the drug. After a suitable period of chronic treatment, the drug is abruptly withdrawn and the subject is observed for abstinence symptoms. Of the 4 methods mentioned, the direct addiction procedure is considered the best (Isbell, 1948), for it yields information in regard to psychic dependence, as well as to tolerance and physical dependence. The Lexington group has also investigated in human subjects the addiction liability of various central nervous system depressants, particularly barbiturate (Isbell, Altschul et al., 1950) and alcohol (Isbell et al., 1955). These experiments have consisted mainly of direct addiction studies.

According to Kolb and Du Mez (1931) and Seevers (1948) the monkey is probably the best infrahuman species to use for analgesic addiction liability experiments, because the signs of abstinence in this species are very similar to those observed in man. The abstinence symptoms in the monkey have been described in detail by Seevers (1936).

A large number of experimental addiction studies have been performed on the dog. The symptoms of morphine addiction and withdrawal in this species are also similar to those of man and have been described by Plant and Pierce (1928). Seevers and Tatum (1931) reported that dogs chronically intoxicated with barbiturate showed tolerance and signs of abstinence, including convulsions. Fraser and Isbell (1954) confirmed these observations and further reported a canine type of delirium during barbiturate abstinence.

There have been but few addiction liability studies in which cats have been employed as test animals. Those that have been reported indicate conflicting results. For example, Gold (1929) and Eddy and Himmelsbach (1936) reported the development of tolerance to the excitatory effects of morphine in the cat, whereas Tatum, Seevers, and Collins (1929) reported increased rather than decreased excitement. Gold (1929) also reported that cats chronically treated with morphine showed drowsiness and loss of appetite as the only signs of abstinence. Recently, Tavat and Akçasu (1956) studied the excitant effects of a series of analgesics on cats. They reported a parallel relation between the ability of

the drugs to cause addiction and the ability of an acute dose to produce excitation in cats, and suggested that the intensity of the excitant effect of new analgesic compounds might be used as an indication of their relative addiction liability.

The rabbit has been used not infrequently for addiction studies. Although numerous investigators reported the development of tolerance to morphine in this species, Tatum and co-workers (1929) and Eddy (1941) claimed that in rabbits, tolerance to the depressant effect of morphine either develops only to a slight extent or not at all. The information regarding withdrawal symptoms in rabbits is also conflicting (Eddy 1941). Since the rabbit shows a gradual decrease in the hyperglycemic response to repeated injections of analgesics and temporary hyperglycemia during withdrawal, Phatak, Maloney and David (1948) suggested that this response might be useful for estimating addiction potentialities of analgesic compounds.

Barlow (1932) described a method for evaluating in rats the tranquilizing potency of a series of narcotic analgesics. This procedure was adapted by Himmelsbach and co-workers (1935) and modified by Eddy and Himmelsbach (1936) for addiction studies. The technic consists of lashing rats in the supine position and measuring the amount of struggle (irritability) before and after a daily dose of drug. Tolerance is indicated by an increase in the post-injection struggle during successive weeks of chronic drug administration; addiction (physical dependence) is indicated

by an increase in the pre-injection struggle. Himmelsbach and co-workers (1935) detected tolerance and physical dependence to a number of analgesics, including morphine, by this technic. However, Shideman and Seevers (1941) were unable to reproduce the results of Himmelsbach and associates during chronic morphine treatment of 8 weeks duration. Stanton (1936) employed Barlow's procedure to determine the effects of daily barbiturate administration. He reported that daily injection of up to 23 mg/kg of sodium phenobarbital or 36 mg/kg of sodium pentobarbital did not result in addiction (physical dependence) and that slight tolerance developed only to the largest dose of pentobarbital administered. Seevers (1954) questions the significance of results obtained in rats.

Mice have been little used in addiction liability studies. Daily injection of morphine in white mice results in a shorter duration (Eddy, 1941) and also a decrease in incidence (Nadzel, 1937) of the Straub response. Recently, McQuarrie and Fingl (1958) employed electroshock and chemoshock procedures to detect alterations in central nervous system excitability in mice following acute and chronic ethanol administration. Chronic ethanol administration decreased central nervous system excitability, as indicated by an increase in seizure threshold, whereas, abrupt withdrawal of ethanol increased excitability, as indicated by a decrease in seizure threshold. These investigators suggested that these technics might be useful in the study of tolerance and physical dependence to central nervous system depressants.

II. STATEMENT OF THE PROBLEM

Considerable evidence has accumulated which indicates that large doses of meprobamate (Miltown, Equanil) may induce addiction to this drug. Barsa and Kline (1956) reported that 6 of 25 schizophrenic patients, who received meprobamate (2400 mg/day) for 9 months, had convulsions following abrupt withdrawal of the drug. Lemere (1956) noted that a patient who had been taking 6.4 g of meprobamate daily for a month experienced a seizure 10 hours after discontinuing medication. Tucker and Wilensky (1957) administered meprobamate to 32 schizophrenic patients for 12 weeks (1600 mg/day for the first 2 weeks, 3200 mg/day for the next 2 weeks, and 4800 mg/day for the remainder of the experimental period) and reported that 2 of the patients had grand mal seizures when the therapy was abruptly terminated. Ewing and Fullilove (1957) slowly withdrew meprobamate (by diminishing the dose over 5 days) from a patient who had been taking 4000 to 8000 mg of the drug daily for several months and observed twitching of the leg during the period of withdrawal. The patient, who was formerly addicted to papaveretum (Pantopon), considered withdrawal symptoms of meprobamate worse than those of opium alkaloids.

In view of the above clinical reports, it seemed important to study in laboratory animals the addiction potentiality of meprobamate and other related agents. Seevers (1948) and Isbell (1948) have emphasized the difficulty of evaluating psychic dependence in laboratory animals and have pointed out that addiction

studies in subhuman species must necessarily be limited to the determination of tolerance and physical dependence. Since McQuarrie and Fingl (1958) have reported that electroshock and chemoshock procedures can be employed to demonstrate physical dependence to alcohol in mice, it was thought important to ascertain whether these technics could be used as a measure of tolerance and physical dependence to other agents in this species. It was appreciated that negative results obtained with these technics would not rule out the possibility that addiction liability exists. Positive results, on the other hand, would be highly suggestive that the agent should be held suspect until clinical trial proves otherwise.

Because both meprobamate and phenaglycodol (Ultran) are substituted diols and have similar pharmacological activities in experimental animals (Berger, 1954; Slater et al., 1956) and in view of the lack of evidence that the latter drug is capable of inducing tolerance and physical dependence, it seemed desirable to include phenaglycodol in this study.

Since both meprobamate and phenaglycodol are known to increase seizure threshold (Chin, 1957) and are known to have anticonvulsant activity (Perlstein, 1956; Gruber and Mosier, 1957), it was thought important also to include an agent known to decrease seizure threshold. Hence, promazine, which has been reported to decrease minimal electroshock seizure threshold (Tedeschi et al., 1958; Fink, 1958) and to induce seizures in

susceptible patients during therapy (Hankoff et al., 1957; Barsa and Kline, 1957; Voegele and May, 1957; Kurtzke, 1957; and Fazekas et al., 1957), was investigated to ascertain whether chronic administration of this drug and its abrupt withdrawal would evoke abstinence symptoms opposite to those observed upon the withdrawal of meprobamate and phenaglycodol.

In view of the apparent role of electrolyte metabolism on the excitability of the central nervous system of laboratory animals (Davenport, 1949; Woodbury, 1952; Woodbury and Davenport, 1949; Woodbury et al., 1957; Timiras et al., 1954; Swinyard et al., 1955; Timiras et al., 1955; Timiras and Woodbury, 1956) the brain electrolyte concentration of meprobamate- and promazine-treated mice were determined in an effort to elucidate further, if possible, the mechanism of tolerance and physical dependence which follows chronic drug administration.

III. GENERAL PROCEDURES

The following procedures were employed in all experiments. Adult male albino mice obtained from the Carworth Farms (CF no. 1 strain) were used as experimental animals. They were maintained on Rockland Mouse Diet and were allowed free access to food and water except for the short periods they were removed from their cages for testing.

The low-frequency electroshock seizure threshold (EST) was determined in mice by means of a Grass stimulator (model (S4B). The stimulus parameters employed were described by Brown and co-workers (1953) and consist of unidirectional pulses of 0.2 millisecond duration delivered for 3 seconds at a frequency of 6 pulses per second. A drop of sodium chloride solution (0.9%) was instilled into each conjunctival sac, the electrodes were held in contact with the corneas and the stimulus was administered. The animal was then observed for signs of a seizure; a "stunning" effect in which a mouse remains motionless for 7 seconds was taken as a minimal positive response. The experimental design of McQuarrie and Fingl (1958) was employed to study central nervous system excitability. For each determination 2 groups of mice were used: a drug-treated group and a control group. Mice were shocked at various voltages selected by the staircase procedure (Finney, 1952); the voltage required to evoke convulsions in 50% of each group was determined and the 95% fiducial limits were calculated by the method of

Litchfield and Wilcoxon (1949). The results, presented as the electroshock seizure threshold ratio (threshold of drug group/ threshold of control group), were computed by the method described in Appendix B.

Pentylenetetrazol seizure threshold (PST was determined in mice by the technic of Orloff and co-workers (1949) as modified by McQuarrie and Fingl (1958). The convulsant solution, composed of 0.5% pentylenetetrazol, 0.9% sodium chloride, and 10 ug/ml of heparin sodium, was infused into the tail vein at a constant rate of 0.005 ml per second by means of the apparatus designed by Gabardi and Esplin (1957) until the mouse exhibited persistent clonus for 3 seconds. The body weight and the pentylenetetrazol infusion time (the time in seconds from the start of the infusion until the endpoint was observed) were recorded to the closest 0.5 g and 0.5 second, respectively, for each animal. The data obtained for groups of drug-treated and control mice were evaluated by analysis of covariance (Finney, 1952). The results, presented as the pentylenetetrazol seizure threshold ratio (threshold of drug group/threshold of control group), were computed by the method described in Appendix B.

Since the present studies were planned to determine whether tolerance develops during the chronic administration of the candidate drugs and whether abrupt withdrawal is followed by alteration of the excitability of the central nervous system, high dose levels of drugs were employed. The doses selected produced defi-

nite toxicity (e.g. ataxia), but not to a level which prevented the animals from eating. Deneau and co-workers (1954) and Seevers (1954) have emphasized that to provide optimal conditions for the development of tolerance and physical dependence it is necessary to maintain a high tissue concentration of drug continuously by adjusting the frequency of drug administration according to the duration of action. Thus, in the studies reported herein the drugs were administered at intervals corresponding to the duration of drug action as determined by toxicity studies or by the EST test. Meprobamate and phenaglycodol were suspended in 6% acacia solution, whereas promazine was dissolved in distilled water. All test drugs were given orally in concentrations such that 10 ml/kg body weight of the suspension or solution contained the appropriate dose of drug.

The threshold altering effects of the test agents were determined at the time of peak drug action as ascertained by the EST or PST test. The effects of withdrawal on central nervous system excitability were determined by these tests at time intervals corresponding to the estimated duration of drug action and, subsequently, at intervals of 24 hours and 48 hours for approximately 1 week.

IV. THE EFFECTS OF CHRONIC AND ACUTE ADMINISTRATION OF PSYCHOPHARMACOLOGICAL AGENTS ON CENTRAL NERVOUS SYSTEM EXCITABILITY OF MICE

A. The Effects of Meprobamate (Miltown, Equanil) on Central Nervous System Excitability of Mice

1. Introduction

The well-known side effects and potential "behavioral toxicity" (Kline, Barsa, and Gosline, 1955; Dickel and Dixon, 1957) of the so-called "tranquilizing agents" justify concern over their indiscriminate use. The added possibility that some of these drugs might also possess addiction liability was suggested by the original clinical reports of Barsa and Kline (1956) and Lemere (1956). These reports indicated that tolerance develops to the effects of meprobamate (Miltown, Equanil) and that abrupt withdrawal in patients who have chronically ingested large doses of the drug may precipitate nervousness, insomnia, and convulsions. In view of these reports, the effects of the chronic administration of this agent and its abrupt withdrawal on the excitability of the central nervous system of mice were studied.

2. Methods

Chronic administration of meprobamate (EST test). One hundred mice, 25 to 35 g in weight, were randomly divided into 2 equal groups. Meprobamate was administered orally as a suspension in 6% acacia solution. The drug was initially given to 1 group of mice in a total daily dose of 1200 mg/kg (200 mg/kg at 8 a.m., 12 noon, 4 p.m., and 8 p.m., and 400 mg/kg at midnight) for 6 days.

Following the 8 a.m. treatment on day 7, each dose was increased by 50% to make a total daily dose of 1800 mg/kg; this higher dose level was continued for 10 days, except as indicated in the legend to figure 1. The other group served as a control and was given the requisite volume of 6% acacia solution at the appropriate intervals. Low-frequency electroshock seizure threshold ratios were determined at the times indicated in the legend to figure 1.

Acute administration of meprobamate (EST test). Eighty mice, 22.0 to 36.5 g in weight, were randomly divided into 2 equal groups. One group was given orally 300 mg/kg of meprobamate, and the other group the requisite volume of 6% acacia solution; the EST ratio was determined at selected times after drug treatment. This procedure was repeated at intervals of 3 or 4 days in the same groups of mice until EST ratios were established 1/2, 1, 2, 3, and 4 hours after drug administration.

Chronic administration of meprobamate (PST test). Twenty-six mice, 23.5 to 30.0 g in weight, were randomly divided into 2 equal groups. The dose schedule for 1 group of mice was identical to that described for the chronic study which employed the EST test, except that the initial dose level was given for 7 days and each dose was increased 50% following the 8 a.m. treatment on day 8 instead of on day 7; also, the larger daily dose was continued for only 7 days as indicated in figure 2. The other group served as a control and was given the requisite volume of 6% acacia solution. Minimal pentylenetetrazol seizure threshold ratios were determined at the times indicated in the legend to figure 2.

Acute administration of meprobamate (PST test). Forty-eight mice, 24.0 to 34.5 g in weight, were randomly divided into 4 equal groups. Two groups were given orally a single dose of 300 mg/kg of meprobamate, and the other two groups the requisite volume of acacia solution. Pentylenetetrazol seizure threshold ratios were determined at selected times after drug administration. This procedure was repeated at intervals of 6 to 10 days until PST ratios were established 1/2, 1, 2, 4, and 6 hours after drug administration.

3. Results

EST test. Administration of a single 300 mg/kg dose of meprobamate to non-tolerant mice increased the electroshock seizure threshold more than 10 fold 1/2 hour after drug treatment (see inset in figure 1), whereas, after chronic treatment for 6 days with 1200 mg/kg/day and then for 10 days with 1800 mg/kg/day, this same dose of drug increased the threshold only 2.5 fold (see A, day 13, figure 1). Thus, tolerance develops to the EST-raising effect of meprobamate. Figure 1 also shows that 4 hours after the 8 a.m. dose on day 12 and 4 hours after drug withdrawal on day 17 (see B and E, figure 1), the EST in mice chronically treated with meprobamate was somewhat less than 0.8 that of control animals. Hyperexcitability was also observed 8 hours after the double midnight dose on day 13 and 8 hours after the 8 a.m. dose on day 14 (see C and D, figure 1). Since EST was not reduced 4 hours after the administration of a single 300 mg/kg dose of meprobamate to non-

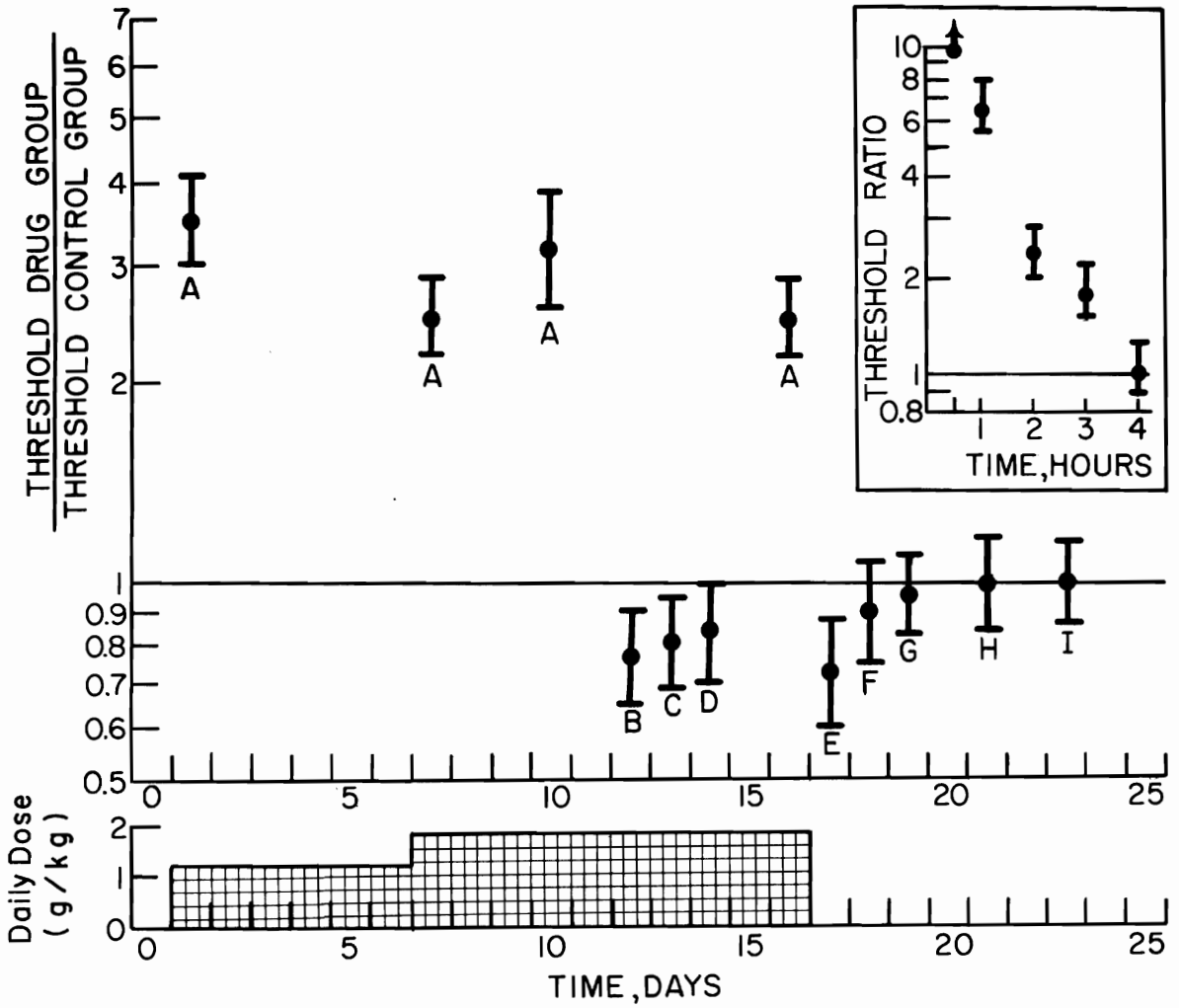

Figure 1. Effects of chronic administration of meprobamate on threshold for low-frequency electroshock seizures in mice. Time in days is indicated on the abscissa. Seizure threshold ratio (threshold drug group/threshold control group) is shown on the upper ordinate and total daily dose of meprobamate administered is shown on the lower ordinate. Vertical bracketed lines indicate 95% fiducial limits. A,B, and D indicate the ratio at 1/2, 4, and 8 hours, respectively, after the 8 a.m. dose; C indicates the ratio 8 hours after the double midnight dose; E, F, G, H, and I indicate the ratio at 4, 28, 52, 100, and 148 hours, respectively, after the final dose of drug. The inset in the upper right-hand corner illustrates the duration (hours) of the effect of a single dose of meprobamate (300 mg/kg) on low-frequency electroshock seizure threshold in non-tolerant mice.

tolerant animals (see inset in figure 1), the withdrawal hyper-excitability must be attributed to the chronic administration of this drug.

PST test. As illustrated in figure 2, tolerance also developed to the PST-raising effect of meprobamate. This was demonstrated by the fact that the PST was increased 4.5 fold in non-tolerant mice 1/2 hour after drug treatment (see inset, figure 2), whereas, after chronic treatment for 7 days with 1200 mg/kg/day and then for 5 days with 1800 mg/kg/day, the same dose of drug increased the threshold only 2.9 fold (see A, day 13, figure 2). Figure 2 also shows that 4 hours after the 8 a.m. dose on day 15, the PST ratio in chronically-treated animals was only 0.82. Since the threshold ratio after the administration of a single dose of 300 mg/kg to non-tolerant animals was 1 (see inset, figure 2), the reduction in the PST ratio observed in the chronically-treated animals must be attributed to the abrupt termination of meprobamate administration.

4. Discussion

The data obtained in these studies indicate that tolerance and physical dependence develop to the chronic administration of large doses of meprobamate to mice. Tolerance is shown by the fact that the EST-raising effect of 300 mg/kg of meprobamate is reduced by at least 75% and the PST-raising effect reduced by approximately 33% in animals receiving the drug chronically. Furthermore, it was noticed that meprobamate-induced ataxia was

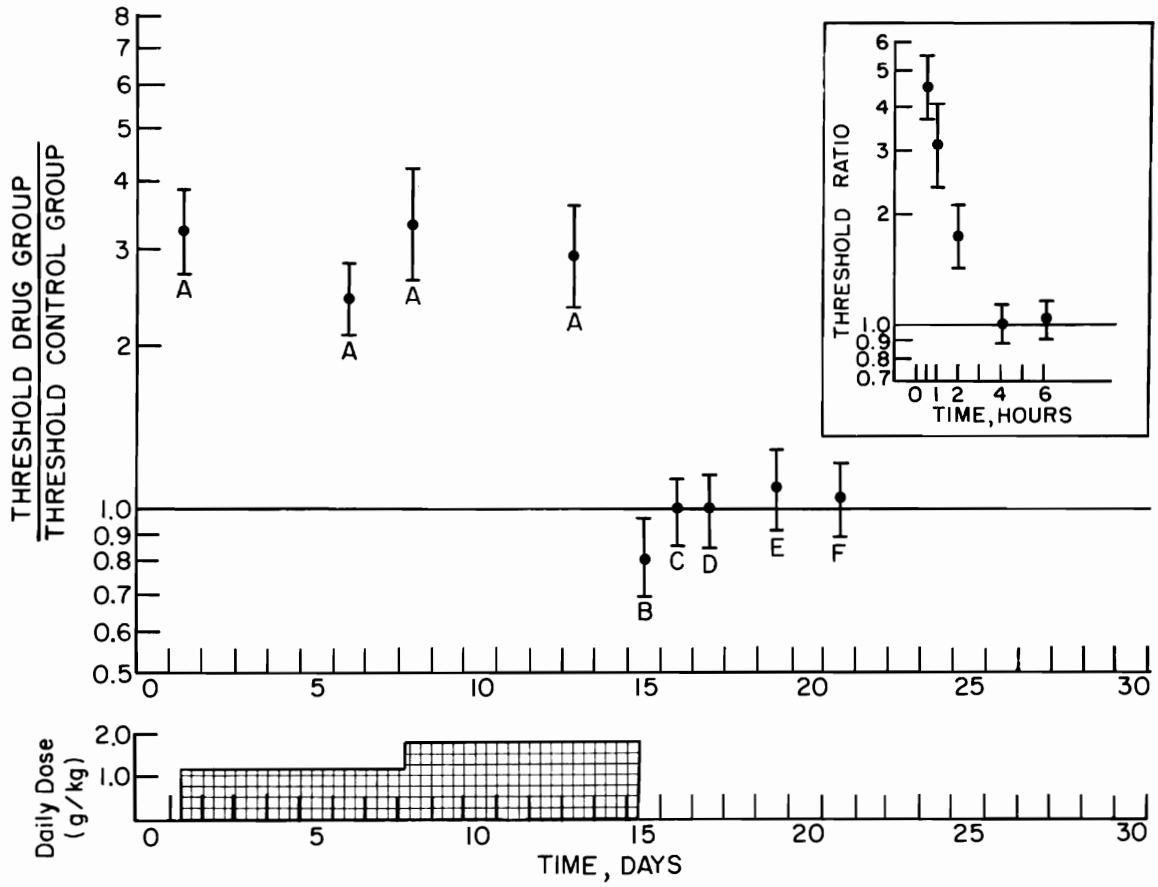

Figure 2. Effects of chronic administration of meprobamate on threshold for pentylenetetrazol seizures in mice. Time in days is indicated on the abscissa. Seizure threshold ratio (threshold drug group/threshold control group) is shown on the upper ordinate and total daily dose of meprobamate administered is shown on the lower ordinate. Vertical bracketed lines indicate 95% fiducial limits. A indicates the ratio 1/2 hour after the 8 a.m. dose; B, C, D, E, and F indicate the ratio at 4, 28, 52, 100, and 150 hours, respectively, after the final dose of drug. The inset in the upper right-hand corner illustrates the duration (hours) of the effect of a single dose of meprobamate (300 mg/kg) on pentylenetetrazol seizure threshold in non-tolerant mice.

less pronounced in the treated animals as drug administration was continued. Physical dependence is demonstrated by the fact that withdrawal hyperexcitability became manifest 4 hours after the final dose of drug; the EST- and PST-ratios of meprobamate-treated mice was only 0.74 (see E, figure 1) and 0.82 (see B, figure 2), respectively. Also, it was observed that after approximately 1 week of chronic meprobamate administration the drug-treated mice manifested a pronounced degree of prein-jection irritability and were relatively more difficult to intu-bate than the control animals. Since neither the EST nor the PST was reduced 4 hours after the single administration of the same dose of meprobamate to non-tolerant animals (see insets in figure 1 and figure 2), it must be concluded that the reduction in threshold represents withdrawal hyperexcitability attribu-table to the chronic administration of this drug.

B. The Effects of Phenaglycodol (Ultran) on Central Nervous System Excitability of Mice

1. Introduction

Studies on meprobamate indicate that tolerance and withdrawal hyperexcitability result from chronic administration of large doses of this drug to mice, as measured by electroshock and chemoshock technics. Because both meprobamate and phenaglycodol (Ultran) are substituted diols and have similar pharmacological activities in experimental animals (Berger, 1954; Slater et al., 1956), it was of interest to determine the effects of chronic administration of large doses of phenaglycodol and of its subsequent abrupt withdrawal on the central nervous system excitability in mice. In the absence of positive experimental and clinical evidence that this drug is capable of inducing tolerance and physical dependence, it was anticipated that such a study might be of value in predicting the effects of abrupt withdrawal of phenaglycodol in patients who have been chronically ingesting large doses of the drug.

2. Methods

Chronic administration of phenaglycodol (EST test). A population sample of mice, 21.0 to 32.0 g in weight, were randomly divided into 2 groups of 50 animals each. Phenaglycodol was administered orally as a suspension in 6% acacia solution. The drug was initially given to 1 group of mice in a total daily dose of 600 mg/kg (200 mg/kg at 7 a.m., 3 p.m., and 11 p.m.) for 6 days.

Following the 7 a.m. treatment on day 7, each dose was increased by 50% to make an average total daily dose of 900 mg/kg; this higher dose level was continued for 11 days, as indicated in figure 3. The other group served as a control and was given the requisite volume of acacia at corresponding intervals. Low-frequency electroshock seizure thresholds were determined for both groups at the times indicated in the legend to figure 3, and the EST ratio calculated.

Acute administration of phenaglycodol (EST test). A population sample of mice, 26.0 to 35.5 g in weight, were randomly divided into 2 groups of 40 animals each. One group was given orally 300 mg/kg of phenaglycodol, and the other group was given the requisite volume of acacia solution. The EST of both groups was determined at a selected time after drug treatment and the EST ratio calculated. This procedure was repeated at intervals of 3 or 4 days in the same groups of mice until EST ratios were obtained 1, 2, 4, 8, 12, 16, and 25 hours after drug administration.

Chronic administration of phenaglycodol (PST test). A population sample of 30 mice, 21.0 to 28.0 g in weight, were randomly divided into 2 equal groups. Phenaglycodol was initially given to 1 group in a total daily dose of 600 mg/kg (200 mg/kg at 8 a.m., 4 p.m., and midnight) for 8 days. On day 9 each dose was increased by 50% to provide a total daily dose of 900 mg/kg; this higher dose level was continued for 9 days, as indicated in figure 4. The other group served as a control and was given the requisite

volume of acacia solution at the appropriate intervals. The PST of both groups were determined at the times indicated in the legend to figure 4, and the ratios calculated.

Acute administration of phenaglycodol (PST test). A population sample of 48 mice, 25.0 to 34.0 g in weight, were randomly divided into 4 equal groups. Two groups were given orally a single dose of 300 mg/kg of phenaglycodol and the other 2 groups the requisite volume of acacia solution. The PST of the 4 groups were determined at a selected time after drug treatment, and the PST ratio calculated. This procedure was repeated at intervals of 6 and 8 days until PST ratios were obtained 1, 4, 8, and 12 hours after drug administration.

3. Results

EST test. A single 300 mg/kg dose of phenaglycodol in mice elevated the electroshock seizure threshold 3.7 fold 1 hour after drug administration to non-tolerant mice (see inset, figure 3), whereas, after chronic treatment for 6 days with 600 mg/kg/day and then for 11 days with 900 mg/kg/day, this same dose of drug elevated the EST only 1.9 fold (see A, day 16, figure 3). Thus, tolerance develops to the EST-raising effect of phenaglycodol.

Figure 3 also shows that 8 hours after the 11 p.m. dose on day 11, 16 hours after the 3 p.m. dose on day 13 and 8 hours after the final dose on day 17 (see B, C, and D, figure 3), the EST in phenaglycodol-treated mice was only 0.81, 0.84, and 0.85, respectively, that of control animals. Since the seizure threshold 8

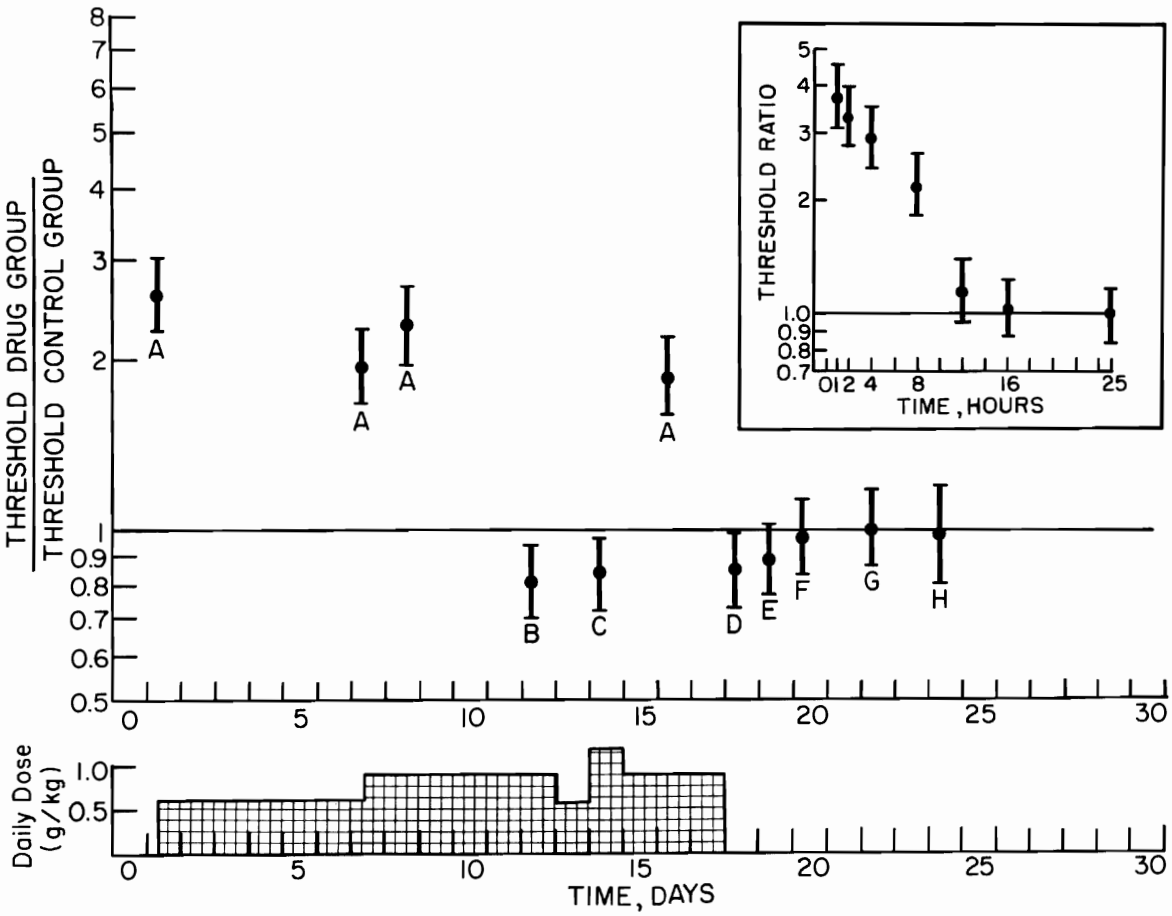

Figure 3. Effects of chronic administration of phenaglycodol on threshold for low-frequency electroshock seizures in mice. Time in days is indicated on the abscissa. Seizure threshold ratio (threshold drug group/threshold control group) is shown on the upper ordinate and total daily dose of phenaglycodol administered is shown on the lower ordinate. Vertical bracketed lines indicate 95% fiducial limits. A indicates the ratio 1 hour after the 7 a.m. dose; B indicates the ratio 8 hours after the 11 p.m. dose; C indicates the ratio 16 hours after the 3 p.m. dose. Following the 16-hour interval, the animals were given a double dose of drug in order to maintain the average daily dose of 900 mg/kg. D, E, F, G, and H indicate the ratio at 8, 32, 56, 104, and 152 hours, respectively, after the final dose of drug. The inset in the upper right-hand corner illustrates the duration (hours) of the effect of a single dose of phenaglycodol (300 mg/kg) on low-frequency electroshock seizure threshold in non-tolerant mice.

hours after administration of a single dose of 300 mg/kg to non-tolerant mice was still 2.19 fold that of control animals (see inset, figure 3), the reduction in threshold observed in the tolerant animals must be attributed to the chronic administration of phenaglycodol.

PST test. The acute administration of 300 mg/kg of phenaglycodol to non-tolerant mice increased the PST 7.4 fold 1 hour after oral intubation (see inset, figure 4), whereas, after chronic treatment for 8 days with 600 mg/kg/day and then for 6 days with 900 mg/kg/day, this same dose of drug elevated the PST only 4.7 fold (see A, day 15, figure 4). Thus, tolerance develops to the PST-raising effect of phenaglycodol.

Figure 4 also illustrates that 8 hours after the midnight dose on day 12 and 12 hours after the midnight dose on day 20, PST ratios in the chronically-treated mice were only 0.82 (see B and D, figure 4). Since the PST ratio was 2.3 in non-tolerant mice 8 hours after the administration of a single dose of 300 mg/kg and was normal at the 12-hour interval (see inset, figure 4), the reduction in seizure threshold observed in the chronically treated mice must be attributed to the abrupt withdrawal of phenaglycodol.

4. Discussion

The data presented in these studies indicate that tolerance and physical dependence develop to the chronic administration of large doses of phenaglycodol. Tolerance is manifested by the

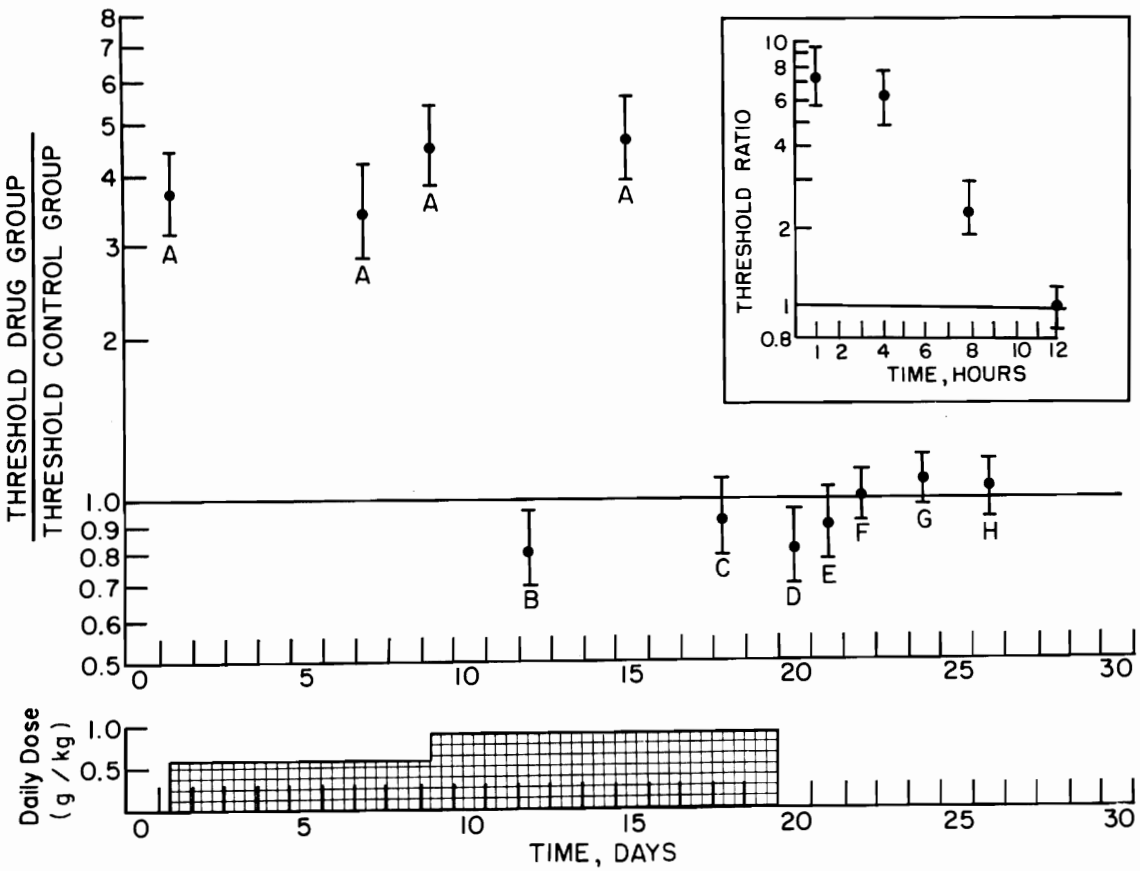

Figure 4. Effects of chronic administration of phenaglycodol on threshold for pentylenetetrazol seizures in mice. Time in days is indicated on the abscissa. Seizure threshold ratio (threshold drug group/threshold control group) is shown on the uppe. ordinate and total daily dose of phenaglycodol administered is shown on the lower ordinate. Vertical bracketed lines indicate 95% fiducial limits. A indicates the ratio 1 hour after the 8 a.m. dose; B and C indicate the ratio 8 hours after the midnight dose; D, E, F, G, and H indicate the ratio 12, 36, 60, 108, and 156 hours, respectively, after the final dose of drug. The inset in the upper right-hand corner illustrates the duration (hours) of the effect of a single dose of phenaglycodol (300 mg/kg) on pentylenetetrazol seizure threshold in non-tolerant mice.

fact that the EST- and PST-raising effect of 300 mg/kg of phenaglycodol is reduced by 50% and 36.5%, respectively. Also, as in the case of chronic meprobamate administration the depressant effect of 300 mg/kg of phenaglycodol appeared less pronounced as the chronic treatment progressed. Physical dependence is demonstrated by withdrawal hyperexcitability; 8 hours after the final dose of phenaglycodol the EST-ratio was only 0.85 (see D, figure 3) and 12 hours after the final dose of drug the PST-ratio was only 0.82 (see D, figure 4). In addition, it was observed that the phenaglycodol-treated mice manifested increased preinjection irritability as drug treatment continued. Since the EST and PST were not reduced 8 hours and 12 hours, respectively, after the acute administration of 300 mg/kg of phenaglycodol to non-tolerant mice (see inset, figures 3 and 4, respectively), it must be concluded that the reduction in both electroshock- and chemoshock-seizure thresholds represent withdrawal hyperexcitability attributable to chronic treatment with this drug.

C. The Effects of Promazine (Sparine) on Central Nervous System Excitability of Mice

1. Introduction

A number of investigators (Hankoff et al., 1957; Barsa and Kline, 1957; Voegele and May, 1957; Kurtzke, 1957; Cares et al., 1957; and Shaw et al., 1959) have reported the occurrence of convulsive seizures in nonepileptic patients during intensive therapy with phenothiazine derivatives. Meszaros and O'Reilly (1956) and Szatmari (1956) concluded that chlorpromazine tends to decrease seizure threshold in epileptic patients and Fazekas and associates (1957) suggested that large doses of chlorpromazine and promazine have the capacity to induce seizures, particularly in susceptible individuals. Fabisch (1957) demonstrated that chlorpromazine in doses of 0.3-0.5 mg/kg body weight, administered intravenously, can increase abnormal activity in the electroencephalograms of epileptic patients; indeed, he suggested that chlorpromazine should be useful as an activating drug in clinical electroencephalographic work.

In animal studies, Essig and Carter (1957) induced convulsions in normal monkeys by the chronic administration of 44-77 mg/kg of chlorpromazine. Das and co-workers (1955) observed that the intravenous injection of chlorpromazine (0.2-0.5 mg/kg) in cats with midbrain transections resulted in transient high voltage spike and wave discharges similar to the electroencephalographic characteristics of petit mal epilepsy; these workers also reported that

the injection of chlorpromazine in cats with the basis pedunculi intact ("pyramidal cats") resulted in occasional convulsions. Kopeloff and co-workers (1955) demonstrated that chlorpromazine, injected intravenously into monkeys with induced-brain lesions, accentuated focal spike and wave electroencephalographic activity and, on some occasions, also induced "spontaneous" seizures. These investigators also found that, after pretreatment with chlorpromazine, smaller doses of pentylenetetrazol were required to provoke seizures in brain-damaged monkeys. Heming and co-workers (1956) observed that the electroshock seizure threshold in rats was lowered after chlorpromazine administration and Tedeschi and co-workers (1958) reported that the phenothiazine derivatives, chlorpromazine, promazine, promethazine, and mepazine produced a significant decrease in minimal electroshock (alternating current) seizure threshold in mice. Fink (1958) demonstrated that chlorpromazine, promazine, and trifluprомazine lower EST.

Because certain central nervous system stimulants, such as amphetamine, induce depression and somnolence upon abrupt withdrawal (Knapp, 1952; Monroe and Drell, 1947) and because certain phenothiazine derivatives increase the excitability of the central nervous system of humans and experimental animals, it was of interest to investigate the effects of chronic administration and abrupt withdrawal of promazine. Promazine was selected for this study because of its comparatively short duration (12-24 hours) of threshold-lowering activity (Fink, 1958). It was thought that the withdrawal effects of a longer-acting phenothiazine derivative (e.g. chlorpromazine) might be masked by the extended action of the drug.

2. Methods

Chronic administration of promazine (EST test). A population sample of 100 mice, 20.0 to 30.0 g in weight, were randomly divided into 2 equal groups. Promazine, as an aqueous solution, was initially administered to 1 group of mice in a total daily dose of 50 mg/kg (25 mg/kg at 8 a.m. and 8 p.m.) for 12 days. On day 13 the 8 p.m. dose was omitted in order to provide a 24-hour period of abstinence for testing. On day 14, each dose was increased by 50% to make a total daily dose of 75 mg/kg; this higher dose level was continued for 5 days. The other group served as a control, and was given the requisite volume of water at the appropriate intervals. Low-frequency EST were determined at the times indicated in the legend to figure 5, and the EST ratios calculated.

Acute administration of promazine (EST test). A population sample of mice, 22.5 to 33.0 g in weight, were randomly divided into 2 groups of approximately 50 animals each. One group of mice was given orally a single 37.5 mg/kg dose of promazine; the other group served as a control and was given 10 ml/kg of water. The EST of both groups were determined 4, 48, and 120 hours after oral intubation and the EST ratios calculated. Three days after the 120-hour threshold determination, drug and water treatment were repeated in the same groups of mice and the EST were determined for the 24- and 72-hour intervals and the EST ratios calculated.

Chronic administration of promazine (PST test). A population sample of 30 mice, 25.0 to 28.5 g in weight, were divided into 2 equal groups. Promazine was initially given to 1 group of mice in a total daily dose of 50 mg/kg (25 mg/kg at 8 a.m. and 8 p.m.) for 8 days. Following the 8 a.m. dose on day 8 each dose was increased by 50% so that a total daily dose of 75 mg/kg was given; this higher dose level was continued for 12 days. The other group served as a control and was administered the requisite volume of water at the proper intervals. PST were established at the times indicated in the legend to figure 5, and the PST ratios calculated.

Acute administration of promazine (PST test). Twenty mice, 25.0 to 30.5 g in weight, were randomly divided into 2 groups of 10 mice each. One group was given orally 37.5 mg/kg of promazine and the other group was given the appropriate volume of water. PST was determined in both groups 1 and 24 hours after the drug treatment and the PST ratios calculated. The procedure was repeated in 2 additional groups of mice, 20.5 to 29.0 g in weight, except that the PST were determined 48, 72, 96, and 144 hours after oral intubation.

3. Results

EST test. The acute administration of 37.5 mg/kg of promazine decreased the EST ratio to 0.77, 4 hours after drug treatment (see inset, figure 5); after chronic treatment for 15 days, this same dose of drug decreased the EST-ratio to approximately

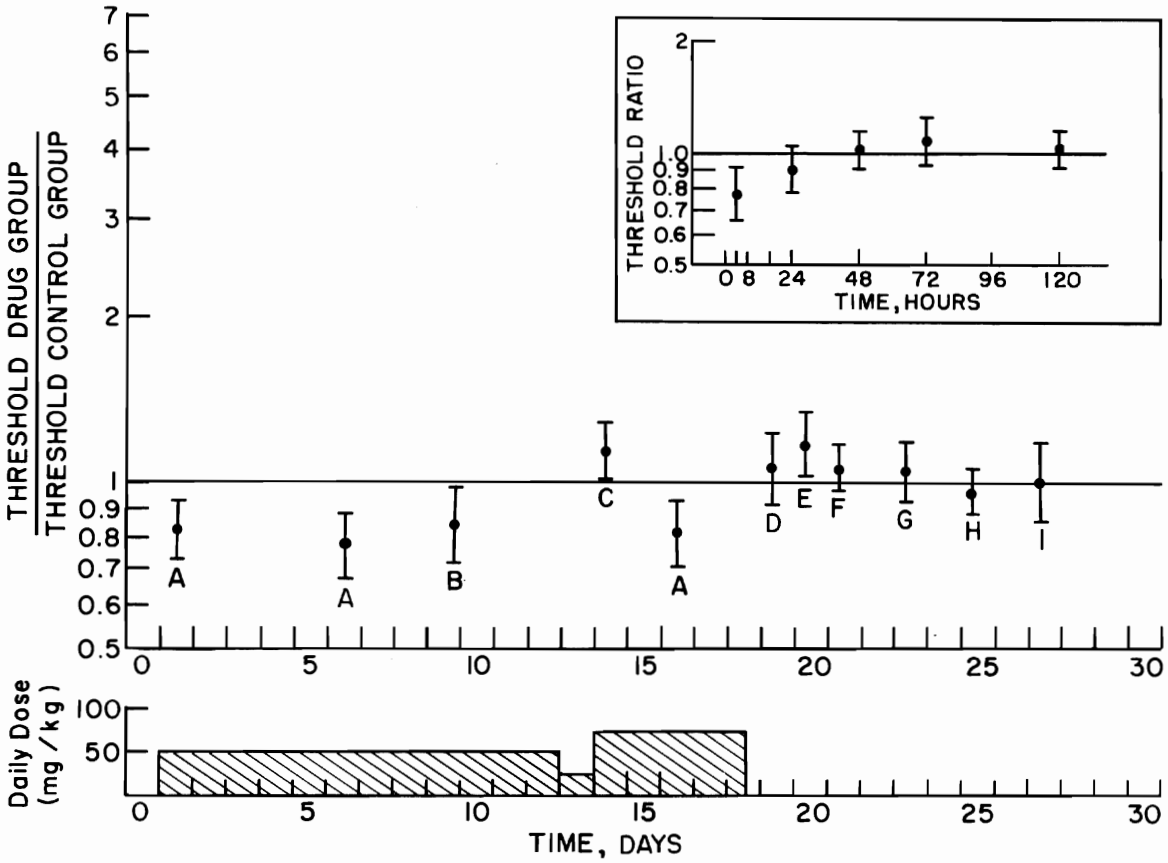

Figure 5. Effects of chronic administration of promazine on threshold for low-frequency electroshock seizures in mice. Time in days is indicated on the abscissa. Seizure threshold ratio (threshold drug group/threshold control group) is shown on the upper ordinate and total daily dose of promazine administered is shown on the lower ordinate. Vertical bracketed lines indicate 95% fiducial limits. A and C indicate the ratio 4 and 24 hours, respectively, after the 8 a.m. dose and B indicates the ratio 12 hours after the 8 p.m. dose. D, E, F, G, H, and I indicate the ratio 24, 48, 72, 120, 168, and 216 hours, respectively, after the final dose of drug. The inset in the upper right-hand corner illustrates the duration (hours) of the effect of a single dose of promazine (37.5 mg/kg) on low-frequency electroshock seizure threshold in mice.

0.81 (see A, day 16, figure 5). Since the 2 ratios are not significantly different, tolerance to the EST-lowering effect of promazine either does not develop or can not be detected by the low-frequency electroshock seizure technic. Figure 5 also shows that on day 14, 24 hours after the 8 a.m. dose (see C, figure 5), and on day 20, 48 hours after the final dose (see E, figure 5), the EST in mice chronically treated with promazine was 1.15 and 1.18, respectively, that of control animals. Since EST was not elevated 24 or 48 hours after the acute administration of a single 37.5 mg/kg dose of promazine (see inset, figure 5), the increase in EST must be attributed to the abrupt withdrawal of this agent.

PST test. Figure 6 shows that tolerance to the pentylenetetrazol seizure threshold-lowering effect of promazine was not detected by the PST test. This was demonstrated by the fact that the PST-lowering effect of promazine was not diminished after chronic treatment for 8 days with 50 mg/kg/day and then for 10 days with 75 mg/kg/day (see A, day 19, figure 6). Figure 6 also indicates that 72 hours after the final dose of promazine (see E, figure 6), the PST ratio in chronically-treated animals was 1.21. Since the PST ratio 72 hours after the administration of 37.5 mg/kg of promazine to the acutely treated animals was 1.02 (see inset, figure 6) the elevation in the PST ratio observed in the chronically treated animals must be attributed to the abrupt cessation of drug treatment.

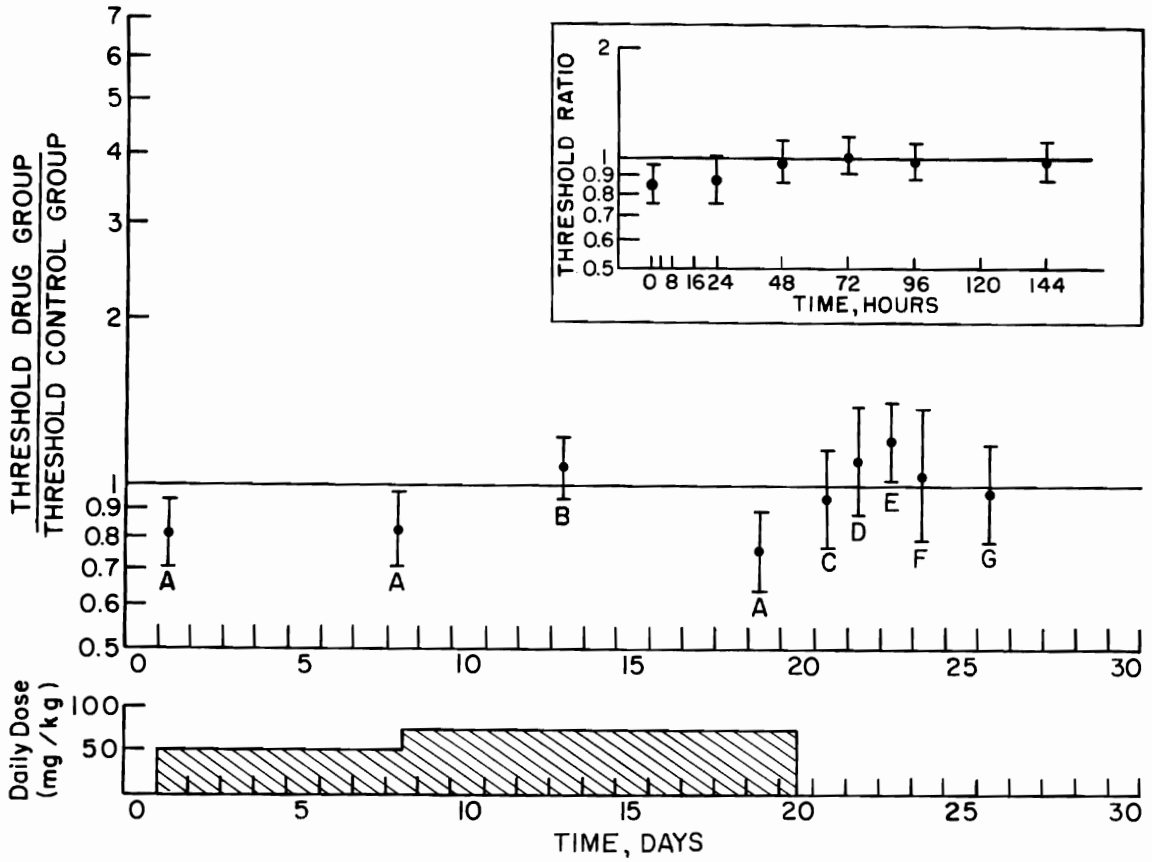

Figure 6. Effects of chronic administration of promazine on threshold for pentylenetetrazol seizure in mice. Time in days is indicated on the abscissa. Seizure threshold ratio (threshold drug group/threshold control group) is shown on the upper ordinate and total daily dose of promazine administered is shown on the lower ordinate. Vertical bracketed lines indicate 95% fiducial limits. A indicates the ratio 1 hour after the 8 a.m. dose and B indicates the ratio 12 hours after the 8 p.m. dose. C, D, E, F and G indicate the ratio 24, 48, 72, 96, and 144 hours, respectively, after the final dose of drug. The inset in the upper right-hand corner illustrates the duration (hours) of the effect of a single dose of promazine (37.5 mg/kg) on pentylenetetrazol seizure threshold in mice.

4. Discussion

The data presented in these studies indicate that although tolerance to the chronic administration of promazine was not detectable in mice by either the EST test or the PST test, a withdrawal effect was observed. Since an acute dose of promazine produces an alteration of either the EST or PST which is opposite to that produced by an acute dose of either meprobamate or phenaglycodol, and since withdrawal of these latter drugs results in hyperexcitability, it is not unreasonable to anticipate that the abrupt withdrawal of promazine after a period of chronic drug administration would result in the appearance of hypoexcitability; indeed this was the case. Hypoexcitability is demonstrated by the fact that the EST-ratio was 1.18, 48 hours after the final dose of promazine (see E, figure 5), and the PST-ratio was 1.21, 72 hours after the final dose of promazine (see E, figure 6). Since neither EST nor PST was elevated in acutely treated mice 48 or 72 hours after a single 37.5 mg/kg dose of promazine, it must be concluded that the elevation in seizure threshold represents withdrawal hypoexcitability attributable to the chronic administration of this agent.

V. THE EFFECTS OF CHRONIC AND ACUTE ADMINISTRATION OF MEPROBAMATE AND PROMAZINE ON ELECTROLYTE DISTRIBUTION IN MICE

A. Introduction

In previous sections of this thesis, it was shown that the chronic administration of meprobamate decreased brain excitability and the abrupt termination of such treatment increased brain excitability. In contrast, the chronic administration of promazine increased brain excitability and the abrupt termination of such treatment decreased brain excitability. Considerable information has been accumulated to indicate that changes in brain Na ratio (extracellular brain Na/intracellular brain Na) can be correlated with changes in brain excitability. Woodbury and coworkers (Woodbury, 1954, 1955, 1958; Woodbury and Timiras, 1957; Woodbury and Esplin, 1959; Timiras et al., 1954; Timiras et al., 1955; Timiras and Woodbury, 1956), employing either alternating-current electroshock seizure threshold or low-frequency electroshock seizure threshold as a measure of brain function, demonstrated that, under a variety of experimental conditions, an increase in the brain Na ratio results in a decrease in brain excitability, whereas a decrease in this ratio results in an increase in brain excitability. Thus, thyroidectomy, hypophysectomy, or treatment with DCA, diphenylhydantoin, or acetazoleamide results in an increase in brain Na ratio and a decrease in brain excitability.

On the other hand, adrenalectomy or treatment with thyroxin or triiodothyronine results in a decrease in the brain Na ratio and an increase in brain excitability. In view of the marked effect of meprobamate and promazine on brain excitability and in view of the apparent role of sodium distribution in brain function, it was thought important to investigate the effects of acute and chronic administration of these agents and their abrupt withdrawal on the distribution of brain electrolyte in mice. It was thought that these studies might contribute to an understanding of the mechanism of tolerance and physical dependence.

B. Methods

A population sample of 270 mice, 20.0 to 28.0 g in weight, were randomly divided into 3 groups of approximately 90 animals each. The first group was chronically treated with meprobamate and the second group was chronically treated with promazine, while the third group served as a control and was given the requisite volume of acacia solution. Meprobamate and promazine were administered daily in the same dose schedules as outlined on pages 21 and 37, respectively, except that no doses were skipped until withdrawal. Hence, the meprobamate-treated mice were initially given 1200 mg/kg/day of drug (200 mg/kg at 8 a.m., 12 noon, 4 p.m., and 8 p.m., and 400 mg/kg at midnight) for 6 days and then 1800 mg/kg/day for an additional 10 days (each dose was increased by 50% following the 8 a.m. dose on day 7). The promazine-treated mice were initially given 50 mg/kg/day of drug

(25 mg/kg at 8 a.m. and 8 p.m.) for 13 days and then 75 mg/kg/day for an additional 5 days (each dose was increased by 50% on day 14). The third group served as a control for the first 2 groups and was given 10 ml/kg of acacia solution on the same dose schedule as the meprobamate-treated animals. The concentration of Cl, Na, K, and water in plasma and brain tissue (cerebral cortex) were determined at the time of peak threshold-raising action of meprobamate (1/2 hour after drug treatment), at the time when hyperexcitability was initially observed following the abrupt withdrawal of meprobamate (4 hours after final drug treatment), at the time when hypoexcitability was initially observed following the abrupt withdrawal of promazine (48 hours after final drug treatment), and at the time when seizure threshold had returned to normal (100 hours after final dose of meprobamate and 168 hours after final dose of promazine). Approximately 250 mice, 17.0 to 38.5 g in weight, were divided into 3 groups for the acute study. Mice acutely treated with a single dose of meprobamate (300 mg/kg) were sacrificed for electrolyte and water determination at intervals of 1/2 and 4 hours following drug treatment and animals acutely treated with promazine (37.5 mg/kg) were sacrificed and examined at intervals of 4 and 48 hours following drug treatment. Control animals were given the requisite volume of acacia solution or water and sacrificed for electrolyte and water determination at appropriate intervals.

Electrolyte determinations were performed on pooled samples comprising the plasma or tissue from 7 to 10 mice. Four such samples were used for each determination. The animals were anesthetized by the intraperitoneal injection of pentobarbital sodium, 70 mg/kg, and blood was drawn from the abdominal aorta. The blood was centrifuged and an aliquot of the supernatant plasma was removed and analyzed for Cl concentration by means of a Cotlove Chloridometer (Laboratory Glass and Instruments Corp.), according to the method of Cotlove and co-workers (1958). The remaining plasma was analyzed for water, Na, and K content. Water content of plasma was determined by difference (wet weight minus dry weight). Aliquots of dried plasma were ashed, solubilized, and analyzed for Na and K concentration by means of a Baird-Atomic Inc. Flame Photometer (model DB4) according to the method described in Appendix A. After exsanguination, the mice were decapitated and the cerebral cortices were removed for water and electrolyte analyses. Water content of brain was determined by difference (wet weight minus dry weight). Approximately 50 mg of dried brain tissue were digested and analyzed for Cl content by the method of Van Slyke and Sendroy (1923), and aliquots of the remaining dried tissue were ashed, solubilized and analyzed for Na and K concentrations by means of a flame photometer. The method of Hastings and Eichelberger (1937) and Manery and Hastings (1939) was employed to calculate intracellular and extracellular electrolyte concentrations. The various technical procedures and methods for calculations are described in detail in Appendix A.

C. Results

The effects of a single dose of meprobamate or promazine on the concentration of electrolytes and water in plasma and brain are summarized in table 1. The administration of a single dose of meprobamate (300 mg/kg) had no significant effect on the concentration of electrolytes or water in plasma or brain ($P>.05$) 1/2 hour (time at which the EST was increased more than 10 fold) or 4 hours (time at which the EST had returned to normal) after drug treatment. Promazine (37.5 mg/kg) produced a significant decrease in the concentration of plasma Na ($P<.05$) 4 hours after drug administration (time at which the EST was decreased approximately 20%), but caused no significant alteration in the concentration of other plasma electrolytes or water or in the concentration of brain electrolytes or water 4 and 48 hours (time at which the EST had returned to normal) after oral intubation.

The effects of chronic administration and abrupt withdrawal of meprobamate or promazine on the concentration of electrolytes and water in plasma and brain are summarized in table 2. The chronic administration of meprobamate altered the concentration of plasma electrolytes only 1/2 hour after the final dose of drug (time at which EST was increased 2.5 fold); thus, the concentration of plasma Na was significantly increased ($P<.05$) and the concentration of plasma Cl significantly decreased ($P<.01$). The chronic administration of meprobamate altered the concentration of brain electrolytes and water 1/2 hour (time at which the EST was

Table 1.

Effects of a Single Dose of Meprobamate (300 mg/kg) or Promazine (37.5 mg/kg) on Water and Electrolyte Concentrations of Plasma and Cerebral Cortical Tissue of Mice[a]

hr after treatment	Procedure	Concentration per kg Plasma				Concentration per kg Moist Cerebral Cortex			
		mEq Na	mEq K	mEq Cl	g H_2O	mEq Na	mEq K	mEq Cl	g H_2O
1/2	Acacia (control)	149.25 ± 0.77[b]	3.55 ±0.08	113.33 ± 2.74	935.4 ± 3.7	42.19 ±1.45	103.48 ± 2.38	28.27 ±0.37	789.1 ± 0.7
1/2[c]	Meprobamate	148.70 ± 1.08	3.36 ±0.10	107.06 ± 1.77	935.1 ± 0.5	42.06 ±1.39	105.39 ± 2.52	29.00 ±0.91	785.5 ± 2.0
4	Acacia (control)	151.90 ± 0.09	3.50 ±0.10	116.64 ± 1.94	933.5 ± 2.6	44.22 ±0.02	108.99 ± 2.07	27.71 ±0.59	787.1 ± 0.5
4	Meprobamate	154.54 ± 1.34	3.53 ±0.30	117.52 ± 2.05	933.5 ± 1.2	43.90 ±0.49	108.94 ± 1.43	28.83 ±0.48	786.0 ± 0.2
4	Promazine	149.18 ± 0.21 (.05-.02)	3.47 ±0.14	114.11 ± 2.78	936.4 ± 1.1	44.77 ±1.10	109.55 ± 1.19	27.66 ±0.30	787.1 ± 0.3
48	Water (control)	150.88 ± 0.59	3.12 ±0.05	114.83 ± 1.56	935.4 ± 0.8	43.52 ±0.40	107.24 ± 1.99	29.71 ±1.11	786.1 ± 0.3
48	Promazine	152.06 ± 0.77	3.39 ±0.13	113.39 ± 0.99	935.7 ± 1.1	43.61 ±0.72	109.46 ± 2.09	27.81 ±0.72	786.3 ± 0.5

[a]Figures in ( ) are P values for differences of results from those of the control group.

[b]± Standard deviation of the mean.

[c]Three samples were employed for this determination; 4 samples were employed for all other determinations.

Table 2.

Effects of Chronic Administration and Abrupt Withdrawal of Meprobamate or Promazine on Water and Electrolyte Concentrations of Plasma and Cerebral Cortical Tissue of Mice[a]

hr after final treatment	Procedure	Concentration per kg Plasma				Concentration per kg Moist Cerebral Cortex			
		mEq Na	mEq K	mEq Cl	g H_2O	mEq Na	mEq K	mEq Cl	g H_2O
1/2	Acacia (Control)	149.55[b] ± 0.54	3.19 ±0.06	121.96 ± 1.72	937.0 ± 2.0	45.91 ±0.55	111.11 ± 1.65	30.08 ±0.36	777.7 ± 1.1
1/2	Meprobamate	151.38 ± 0.51 (.05 -.02)	3.37 ±0.08	111.84 ± 1.89 (.01 -.001)	935.4 ± 3.6	46.97 ±0.36	117.55 ± 1.70 (.05 -.02)	27.44 ±0.54 (.01 -.001)	770.4 ± 1.5 (.01 -.001)
4	Acacia (Control)	152.51 ± 0.46	3.15 ±0.10	113.14 ± 2.65	931.4 ± 2.7	44.84 ±0.83	94.27 ± 2.29	28.48 ±0.52	783.4 ± 1.8
4	Meprobamate	151.53 ± 1.55	3.16 ±0.11	114.69 ± 1.15	924.8 ± 0.6	44.42 ±0.46	90.36 ± 1.98	30.47 ±0.55 (0.5 -0.2)	781.5 ± 2.0
48	Promazine	152.84 ± 0.87	3.26 ±0.09	108.48 ± 1.68	932.7 ± 0.8	44.40 ±1.09	89.93 ± 3.24	29.82 ±1.45	778.5 ± 3.1
100	Acacia (Control)	151.89 ± 1.00	3.68 ±0.11	115.40 ± 2.10	934.4 ± 0.8	44.40 ±0.59	101.08 ± 3.09	28.93 ±0.24	785.2 ± 0.4
100	Meprobamate	150.40 ± 0.63	3.65 ±0.12	114.66 ± 2.46	934.2 ± 1.8	44.75 ±0.29	97.06 ± 1.19	28.08 ±0.49	783.0 ± 0.8
168	Promazine	150.94 ± 0.93	3.46 ±0.02	116.50 ± 1.68	935.8 ± 2.0	44.40 ±1.38	100.13 ± 4.72	27.45 ±1.18	784.3 ± 0.6

[a]Figures in ( ) are P values for differences of results from those of the control group

[b]± Standard deviation of the mean.

increased 2.5 fold) and 4 hours (time at which the EST was decreased approximately 20%) after drug treatment, but had no significant effect on the components studied at 100 hours (time at which EST had returned to normal). Thus, the K concentration of moist brain was significantly increased ($P<.05$) 1/2 hour after the final dose of meprobamate and was somewhat lower than control values at 4 and 100 hours; the Cl concentration was significantly decreased ($P<.01$) at 1/2 hour and was significantly increased ($P<.05$) at 4 hours; and the water concentration was significantly decreased ($P<.01$) at 1/2 hour and was essentially normal thereafter. Promazine tended to decrease the concentration of plasma Cl and brain K 48 hours after administration of the final dose of drug (time at which the EST was increased approximately 20%), but induced no other changes in the concentration of electrolyte or water in plasma or brain at 48 or 168 hours (time at which EST had returned to normal).

The distribution of electrolytes and water in the intracellular and extracellular space was calculated from the data presented in tables 1 and 2 by the method described by Hastings and co-workers (Hastings and Eichelberger, 1937; Manery and Hastings, 1939). Chloride space, which was assumed to be a measure of the extracellular fluid volume, was determined from the concentration of Cl in plasma and brain. Although chloride space is not identical with the extracellular space, numerous investigators (Manery and Hastings, 1939; Winkler et al., 1944; Swinyard, 1949; Nichols et al., 1953; and Cotlove, 1954) have concluded that changes

in the 2 spaces are parallel. Therefore, chloride space offers a reliable index for estimating directional changes in the volume of extracellular fluid.

The effects of a single dose of meprobamate or promazine on the extracellular and intracellular distribution of electrolyte and water in cerebral cortical tissue are summarized in table 3. Except for the effect of meprobamate on brain K ratio at 1/2 hour, brain intracellular Na at 4 hours, and brain Na ratio at 4 hours, a single 300 mg/kg dose of meprobamate or a single 37.5 mg/kg dose of promazine had no significant effect on any of the calculated values for the distribution of electrolytes and water in cerebral cortical tissue. The significant changes ($P<.05$) induced by a 300 mg/kg dose of meprobamate include an increase in brain K ratio at 1/2 hour, a decrease in the concentration of intracellular Na in moist brain and in cellular water at 4 hours, and an increase in brain Na ratio at 4 hours. Although there appear to be changes in the calculated distribution of other electrolytes and water which may be important, the alterations induced were not significant. For example, after the administration of meprobamate the Cl space tended to be increased at 1/2 and 4 hours, the concentration of intracellular Na in moist brain and in cellular water tended to be decreased at 1/2 hour, and the brain Na ratio tended to be increased at 1/2 hour. After the administration of promazine the brain K ratio tended to be decreased at 48 hours.

Table 3.

Effects of a Single Dose of Meprobamate (300 mg/kg) or Promazine (37.5 mg/kg) on Extracellular and Intracellular Distribution of Electrolytes and Water in Cerebral Cortical Tissue of Mice[a]

Hr after treatment	Procedure	Cl space (extracell) g/kg moist brain	Cl-free space (intracell) g/kg moist brain	Intracell Na mEq/kg moist brain	Intracell Na mEq/kg cellular water	Extracell/Intracell Na	Intracell K mEq/kg moist brain	Intracell K mEq/kg cellular water	Intracell/Extracell K
1/2	Acacia (control)	222.1 ± 6.7[b]	566.7 ± 7.0	8.53 ±2.00	14.98 ±3.42	11.82 ±2.52	102.68 ± 2.38	181.20 ± 3.84	50.35 ±0.68
1/2[c]	Meprobamate	241.3 ±12.9	544.2 ±14.9	5.61 ±2.13	10.13 ±4.13	13.74 ±4.93	104.56 ± 2.53	192.46 ± 7.47	56.48 ±2.60 (.05-.02)
4	Acacia (control)	210.7 ± 3.4	576.4 ± 3.5	11.65 ±0.54	20.19 ±0.84	7.70 ±0.35	108.24 ± 2.05	187.84 ± 4.50	52.83 ±1.52
4	Meprobamate	217.6 ± 0.9	568.4 ± 0.7	9.76 ±0.42 (.05-.02)	17.18 ±0.74 (.05-.02)	9.19 ±0.44 (.05-.02)	108.16 ± 1.45	190.29 ± 2.46	54.22 ±4.77
4	Promazine	216.3 ± 4.8	570.8 ± 4.7	12.05 ±0.72	21.12 ±1.34	7.26 ±0.47	108.78 ± 1.23	190.55 ± 0.99	54.33 ±2.26
48	Water (control)	230.3 ±10.5	555.9 ±10.2	9.36 ±1.07	16.78 ±1.78	9.54 ±1.27	105.94 ± 2.13	190.75 ± 4.77	60.36 ±2.21
48	Promazine	218.1 ± 6.3	568.2 ± 6.6	9.97 ±1.32	17.48 ±2.11	9.15 ±0.88	108.71 ± 2.08	191.35 ± 3.19	55.73 ±2.05

[a] Figures in ( ) are P values for differences of results from those of the control group.

[b] ± Standard deviation of the mean.

[c] Three samples were employed for this determination; 4 samples were employed for all other determinations.

The effects of chronic administration and abrupt withdrawal of meprobamate or promazine on extracellular and intracellular distribution of electrolytes and water in cerebral cortical tissues are summarized in table 4. Neither the chronic administration of meprobamate or promazine nor their abrupt withdrawal had any significant effect on the calculated values for the distribution of electrolytes and water in cerebral cortical tissue. Although there appear to be changes in the calculated distribution of other electrolytes and water which may be important, the alterations induced were not significant. For example, after the administration of meprobamate the Cl space tended to be decreased at 4 and 100 hours, the concentration of intracellular Na in moist brain and in cellular water tended to be decreased at 4 hours, and the brain Na ratio tended to be increased at 4 hours. After the administration of promazine the Cl space tended to be increased at 48 hours and tended to be decreased at 168 hours, the concentration of intracellular Na in moist brain and cellular water tended to be decreased at 48 hours, and the brain Na ratio tended to be increased at 48 hours.

Table 4

Effects of Chronic Administration and Abrupt Withdrawal of Meprobamate or Promazine on Extracellular and Intracellular Distribution of Electrolytes and Water in Cerebral Cortical Tissue of Mice

Hr after treatment	Procedure	Cl space (extracell) g/kg moist brain	Cl-free space (intracell) g/kg moist brain	Intracell Na mEq/kg moist brain	Intracell Na mEq/kg cellular water	Extracell / Intracell Na	Intracell K mEq/kg moist brain	Intracell K mEq/kg cellular water	Intracell / Extracell K
1/2	Acacia (control)	219.7 ± 5.0[a]	558.0 ± 4.7	12.60 ±1.08	22.54 ±1.78	6.85 ±0.54	110.40 ± 1.65	197.90 ± 3.47	61.48 ±1.62
1/2	Meprobamate	218.1 ± 4.9	552.3 ± 4.8	13.44 ±0.63	24.32 ±0.97	6.36 ±0.26	116.80 ± 1.69	211.59 ± 4.43	62.07 ±1.88
4	Acacia (control)	223.3 ± 8.3	560.2 ± 6.7	10.13 ±1.88	17.98 ±3.15	9.43 ±1.50	93.56 ± 2.29	166.99 ± 3.10	52.11 ±0.90
4	Meprobamate	233.4 ± 2.6	548.1 ± 4.2	8.13 ±0.54	14.84 ±1.01	10.66 ±0.89	89.60 ± 1.95	163.55 ± 4.30	50.49 ±0.57
48	Promazine	239.1 ± 8.7	539.4 ±11.3	7.20 ±0.92	13.34 ±1.68	12.19 ±1.36	89.14 ± 3.23	165.76 ± 8.78	50.29 ±3.79
100	Acacia (control)	222.8 ± 5.4	562.4 ± 5.3	10.01 ±1.15	17.75 ±1.84	8.93 ±0.72	100.24 ± 3.09	178.24 ± 5.70	47.66 ±1.78
100	Meprobamate	217.9 ± 8.5	565.1 ± 8.7	11.44 ±1.39	20.15 ±2.16	7.87 ±0.86	96.25 ± 1.65	170.78 ± 4.11	46.16 ±1.95
168	Promazine	209.7 ±10.6	574.6 ±11.2	12.28 ±2.43	21.18 ±3.96	8.19 ±1.71	99.40 ± 4.75	172.75 ± 5.44	49.13 ±1.30

[a] ± Standard deviation of the mean.

D. Discussion

The results presented in the preceding section indicate that except for the significant decrease in the concentration of plasma Na 4 hours after promazine, the administration of a single 300 mg/kg dose of meprobamate or a single 37.5 mg/kg dose of promazine induced no marked changes in the electrolyte and water concentration of plasma and brain in the time periods studied. In contrast, electrolyte and water distribution in plasma and brain was markedly altered in the chronically-treated animals 1/2 hour after a dose of meprobamate. No significant alteration in the concentration of electrolytes or water in plasma and brain occurred in the chronically-treated animals 48 and 168 hours after the administration of promazine.

The effects of meprobamate, after a single dose or after chronic treatment, on the EST and on the calculated distribution of electrolytes and water in brain tissue of mice are summarized graphically in table 5. It may be seen that 1/2 hour after the administration of a single dose of meprobamate, the time at which the EST was increased more than 10 fold, the electrolyte pattern was characterized by a significant increase ($P<.05$) of the brain K ratio. Whereas, 4 hours after the administration of a single dose of meprobamate, the time at which the EST was essentially normal, the electrolyte pattern was characterized by a significant increase in the brain Na ratio. In comparison, 1/2 hour after the administration of meprobamate to chronically-treated mice, when

Table 5.

The Comparative Effects of Single-Dose and of Chronic Treatment with Meprobamate on EST and on Brain Electrolyte Distribution in Mice[a]

Type of Treatment	Hr after Treatment	EST Ratio	Plasma Na	Cl Space	Intracellular Na	Brain Na Ratio	Intracellular K	Brain K Ratio
Single Dose	1/2	↑ (>10)	↔	↔	↔	↔	↔	↑
Single Dose	4	↔	↔	↔	↔	↑	↔	↔
Chronic	1/2	↑ (ca. 2.5)	↑	↔	↔	↔	↔	↔
Chronic	4	↓ (ca. 0.8)	↔	↔	↔	↔	↔	↔
Chronic	100	↔	↔	↔	↔	↔	↔	↔

[a]The direction of the arrow indicates the direction of the significant change ($P<.05$). The horizontal arrow indicates that there is no significant change in response; a tendency of the response to increase or to decrease is shown by the appropriate curve in the shaft of the horizontal arrow.

the EST was increased 2.5 fold, the electrolyte pattern was characterized by a significant increase in the concentration of plasma Na. At the 4-hour period, in the chronically-treated animals, when the EST was decreased by approximately 20%, and at the 100-hour period when the EST was essentially normal, there were no significant alterations in the electrolyte and water distribution in brain. It is obvious from table 5 that there is no consistent correlation between brain electrolyte alterations and brain excitability. Indeed, the increased brain Na ratio 4 hours after a single dose of meprobamate, at a time when the EST was essentially normal, appears to be contradictory to the accepted concept that an increased brain Na ratio is associated with an increase in the EST (decreased brain excitability). Also, in the chronically-treated animals, 1/2 hour after the administration of meprobamate, at a time when the EST was increased 2.5 fold, there was no change in the brain Na ratio and 4 hours after the administration of meprobamate, at a time when the EST was decreased approximately 20%, the brain Na ratio tended to be increased. Woodbury (1958) has demonstrated that the rate of electrolyte exchange from plasma to brain extracellular space is delayed by the blood-brain barrier. Hence, in view of the acute nature of the electrolyte studies after meprobamate treatment, i.e. the relatively short time interval between drug adminis-

tration and sacrifice of the animals, it is probable that the seemingly anomalous brain Na ratio at the 4-hour time periods may be due to a lag in the movement of Na across the blood brain barrier and that the brain Na ratio observed at the 4-hour periods may merely reflect brain function during the earlier intervals of decreased brain function. Comparison of the brain Na ratio-EST relation between single-dose treatment and chronic treatment indicates a trend, which suggests that the chronic administration of meprobamate tends to diminish the changes in the brain Na ratio at the 1/2- and 4-hour time periods. It is tempting to speculate that this action of meprobamate on the brain Na ratio may be associated with the development of tolerance to the threshold-raising effect of this drug.

The effects of promazine, after a single dose or chronic treatment, on the EST and on the calculated distribution of electrolytes and water in brain tissue of mice are illustrated in table 6. It may be seen that although 4 hours after a single dose of promazine the EST was decreased approximately 20% and 48 hours after abrupt withdrawal of the drug from chronically-treated animals the EST is increased approximately 20%, there were no significant alterations in the brain electrolyte pattern. However, there was a tendency for the brain Na ratio to be increased 48 hours after the withdrawal of promazine, at a time when the

Table 6.

The Comparative Effects of Single-Dose and of Chronic Treatment with Promazine on EST and on Brain Electrolyte Distribution in Mice[a]

Type of Treatment	Hr after Treatment	EST Ratio	Plasma Na	Cl Space	Intracellular Na	Brain Na Ratio	Intracellular K	Brain K Ratio
Single Dose	4	↓ (ca. 0.8)	↓	↔	↔	↔	↔	↔
Single Dose	48	↔	↔	↔	↔	↔	↔	↔
Chronic	48	↑ (ca. 1.2)	↔	↔	↔	↔	↔	↔
Chronic	168	↔	↔	↔	↔	↔	↔	↔

[a]The direction of the arrow indicates the direction of the significant change ($P<.05$). The horizontal arrow indicates that there is no significant change in response; a tendency of the response to increase or to decrease is shown by the appropriate curve in the shaft of the horizontal arrow.

EST was increased approximately 20%. Although the increase in the brain Na ratio at this time period was not statistically significant, it, nevertheless, suggests that hypoexcitability which results from the abrupt withdrawal of promazine may, at least in part, be associated with an alteration in the brain Na ratio.

Woodbury (1959) is of the opinion that in acute electrolyte studies alterations in the brain K ratio are sometimes useful measures of changes in brain function, whereas under other conditions alterations in the concentration of plasma Na may reflect changes in brain function. Thus, an increase in the brain K ratio or in the concentration of plasma Na is associated with an increase in EST, whereas a decrease in these values is associated with a decrease in EST. It can be observed from tables 5 and 6 that, in general, alterations in the brain K ratio and concentration of plasma Na tend to relate well with changes in the EST. For example, 1/2 hour after a single dose of meprobamate the increased brain K ratio is associated with an increased EST and 1/2 hour after meprobamate in chronically-treated animals the increased concentration of plasma Na is associated with an increased EST (see table 5). Four hours after a single dose of promazine, the decreased concentration of plasma Na is associated with a decreased EST (see table 6).

The accurate interpretation of data obtained from electrolyte studies as presented herein is difficult and may be compromised by the time-curve of the experiments. Woodbury (1958) has succinctly

described some factors which compromise the results of brain electrolyte studies: the role of the blood-brain barrier is usually ignored, calculations are based on the assumption that chloride is a measure of extracellular space without regard to the kinetics of the anion, and no distinction is made between the rapid and slow phase in the extracellular compartment of brain tissue. The half time for equilibration of the rapid phase (plasma and brain extracellular space) is 1 and 1/2 hours and that for equilibration of the slow phase (brain extracellular space and glial tissue) is 24 hours. Therefore, it is obvious that most electrolyte studies reported herein were done while brain water and electrolytes were in an unsteady state.

VI. GENERAL DISCUSSION

The results of the electroshock and chemoshock studies show that meprobamate increases the EST and PST in mice. During chronic treatment, the EST- and PST-raising effect of meprobamate was diminished, a response which indicates the development of tolerance. When chronic drug administration was abruptly terminated the EST and PST were significantly decreased below normal, a response indicative of withdrawal hyperexcitability or physical dependence. Several recent laboratory and clinical reports correlate well with these findings. For example, Essig (1958) administered large daily doses of meprobamate to 4 dogs for 4 to 6 months. Upon abrupt withdrawal of the drug, all animals exhibited convulsions and 3 of these dogs died after 3 to 5 seizures. Greaves and West (1957) reported 2 patients who experienced grand mal seizures after termination of meprobamate medication. Interpretation of the observations reported for 1 case is complicated by the fact that the patient received 25 mg/kg of chlorpromazine 4 times daily following termination of meprobamate therapy, but the remaining patient received no medication during withdrawal. Furthermore, these investigators also observed that therapeutic benefit from meprobamate diminished during drug treatment; this suggests that tolerance develops to the drug. In a double blind study, Haizlip and Ewing (1958) reported that of 47 patients who received 6.4 or 3.2 g of meprobamate daily for 40 days 44 patients exhibited abstinence symptoms when drug therapy was discontinued.

Typical symptoms included insomnia, vomiting, tremors, muscle twitching, overt anxiety, anorexia, and ataxia. Eight patients showed evidence of hallucinations with marked anxiety and tremors, a syndrome similar to delirium tremens, and 3 patients exhibited grand mal seizures.

The electroshock and chemoshock studies revealed that the EST and PST responses to phenaglycodol are qualitatively very similar to those of meprobamate. For example, during chronic treatment the EST- and PST-raising effect of this drug was diminished and when chronic drug treatment was abruptly terminated the EST and PST were significantly decreased below normal. Hence, the results obtained indicate that phenaglycodol is capable of inducing the development of tolerance and withdrawal hyperexcitability or physical dependence. Although there has been as yet no clinical reports describing physical dependence to phenaglycodol, the results of the present study suggest that the drug should be held suspect until adequate clinical evidence proves that it is free from addiction liability. Because of the similarity in the withdrawal hyperexcitability induced in mice by the chronic administration of either phenaglycodol or meprobamate and the evidence that continued ingestion of large doses of meprobamate can cause physical dependence in both animals and man, precautions should be taken to minimize the development of physical dependence to either of these agents and the severity of the withdrawal symptoms. Thus, large doses of phenaglycodol

or meprobamate should be avoided, if possible, and these drugs should be withdrawn gradually, rather than abruptly, from patients who have been ingesting large doses chronically.

The results of the electroshock and chemoshock studies indicate that promazine decreases the EST and PST in mice (increased brain excitability). These findings agree well with clinical reports that certain phenothiazine derivatives, including promazine, increase central nervous system excitability (Hankoff et al., 1957; Barsa and Kline, 1957; Voegele and May, 1957; Kurtzke, 1957; Cares et al., 1957; Fazekas, 1957; and Shaw et al., 1959). In view of the withdrawal hypoexcitability observed in mice when chronic promazine treatment was abruptly terminated, it is possible that mental patients under heavy phenothiazine therapy for an extended period of time may exhibit similar withdrawal hypoexcitability which may be erroneously interpreted as a return of pretreatment symptoms. Such effects could be avoided by gradually withdrawing the drug when the time comes to discontinue therapy.

The effects of a single dose of meprobamate or promazine and of the chronic administration of these same agents on electrolyte and water concentration in plasma and cerebral cortical tissue were studied in an effort to reveal changes which might explain, at least in part, the phenomena of tolerance and physical dependence. Before discussing the data obtained in these studies it is appropriate at this point briefly to review suggested theories for tolerance and physical dependence.

Many theories have been advanced to explain drug addiction, but none is entirely satisfactory and the mechanism of drug tolerance and physical dependence is still obscure. Most of these theories (e.g. antitoxin, cellular immunity, pathobiosis, allergy, replacement of cell constituent, reversible coagulation, dual action theory, etc.) have been reviewed by Tatum and Seevers (1931), Kolb and Himmelsbach (1936), Eddy (1941), Isbell and Fraser (1950) and Wikler (1950). It may be of interest to mention theories that have been presented subsequent to these reviews.

The concept that the development of tolerance is due to an increased ability to metabolize or inactivate a drug has been a controversial one. For example, Gross and Thompson (1940) reported that tolerant and non-tolerant dogs excreted about the same proportion of the administered dose of morphine but the former excreted less "bound" morphine than the latter. This report reopened the consideration that tolerant animals are capable of destroying or inactivating a larger portion of the injected dose of morphine than non-tolerant animals. Subsequently, Zauder (1952) confirmed these findings in rats, and, in addition, demonstrated that liver from tolerant rats have an increased ability to conjugate morphine <u>in vitro</u>. In contrast, Cochin and co-workers (1954) reported no significant difference between the free- and bound-morphine plasma concentration-time curves of tolerant and non-tolerant dogs after subcutaneous injection of the analgesic. They concluded that alterations in

the pattern of morphine excretion do not account for the development of tolerance and physical dependence in dogs. On the basis of similar experiments on dogs and rats, Woods (1954) stated that to date no acceptable evidence has been presented to support the view that altered distribution or fate of morphine is associated with or responsible for the development of tolerance or physical dependence.

Kasé (1956) demonstrated that electroshock and pentylenetetrazol seizures, which enhance the staining action of dye in the central nervous system after intravenous injection, can reduce or eliminate tolerance to the antitussive effect of codeine and morphine for several days. He therefore suggested that tolerance to the antitussive effects of codeine and morphine occurs as the result of a decreased permeability of the blood-brain barrier consequent to the repeated administration of these analgesics. However, he made no mention of the role of the blood-brain barrier on physical dependence.

Axelrod (1956) reported that liver from rats tolerant to morphine loses much of its ability to N-demethylate morphine, dilaudid, and meperidine. Further, he demonstrated a parallelism between ability of analgesics to depress enzyme N-demethylation and their ability to induce tolerance, and observed that nalorphine which blocks the development of tolerance (Orahovats et al., 1953) also blocks the reduction of enzyme activity. On the basis of these observations he suggested that the continuous interaction of narcotic drugs with demethylation enzymes inactivates the latter.

Thus, he reasons that if it is assumed that enzymes which N-demethylate narcotic drugs are very similar to the receptors for these agents, it may be postulated that continued interaction of narcotic drugs with their receptors may inactivate the latter; hence, a decreased response or tolerance to the narcotic may develop as a result of unavailability of receptor sites. In connection with N-demethylation of morphine, Fraser and co-workers (1958) advanced the hypothesis that demethylated morphine, normorphine, opposes the cellular "counter-adaptations" presumably responsible for the morphine abstinence syndrome. However, in testing their hypothesis, they observed that normorphine not only has the ability to suppress morphine abstinence symptoms, but that it is also capable of producing direct physical dependence.

That the phenomenon of drug tolerance occurs at the cellular level has been postulated by a number of investigators. For example, Hatcher and Gold (1929) found no difference in the rate of disappearance of morphine from blood between tolerant and non-tolerant dogs and postulated that tolerance is due mainly to increased cellular resistance. Schmidt and Livingston (1933a) demonstrated that dogs chronically treated with morphine become tolerant to the depressor action of the intravenously injected drug and suggested that this phenomenon is not peculiar to blood vessels, but is shared by vasomotor and respiratory centers and the cerebrum. They also observed (Schmidt and Livingston, 1933b) that an "acute tolerance" could be induced over a period of 3 hours by the administration of a large divided dose of morphine,

and suggested that the underlying cause must be the same in the 2 cases. They postulated that the cause for the development of tolerance is due to a cellular change of an unknown nature, based upon the presence of a concentration of morphine that equals or exceeds a critical level; presumably, it makes little difference whether this critical level is attained by prolonged administration over weeks or months or by repeated massive doses over a short interval of time. As in the case of morphine, alcohol-tolerant animals apparently do not have the ability to increase the rate of metabolism of alcohol (Newman and Cutting, 1935, 1936; Newman and Lehman, 1938) or rate of excretion of alcohol (Gettler and Freireich, 1935). Consequently, Newman and Lehman (1938) suggested that acquired tolerance to alcohol is primarily a tissue tolerance; the cells of the central nervous system develop the ability to function more effectively at a given concentration of alcohol. Recently, McCormick and Kniker (1935) reported that tissues obtained from rats chronically treated with morphine or racemorphan (Dromoran) can grow in tissue cultures containing lethal concentrations of the analgesics. They concluded that cellular tolerance must represent homeostatic changes occurring within the cell. However, tissues from rats chronically treated with sodium amobarbital developed no tolerance to lethal concentrations of the depressant. Also, no dependence to morphine, racemorphan, or sodium amobarbital could be demonstrated in tissue cultures.

The most acceptable theory of tolerance and physical dependence is that they are cellular phenomena involving cells of the central nervous system. Hence, since Woodbury and co-workers (op. cit.) observed that the brain Na ratio (extracellular brain Na/intracellular brain Na) is correlated with central nervous system excitability it was thought important to analyze the electrolyte distribution in brains of mice chronically treated with meprobamate and promazine in an effort to determine whether this ratio might be associated with withdrawal hyperexcitability and withdrawal hypoexcitability. The result of the electrolyte studies presented in table 4 indicate that a correlation between the brain Na ratio and withdrawal hyperexcitability, 4 hours after the abrupt termination of chronic meprobamate administration, could not be established. Indeed, the data showed that 4 hours after the final dose of meprobamate the brain Na ratio tended to be increased (see table 4), at a time interval when the EST had returned to normal (see E, figure 1). Although this observation may appear to contradict the accepted concept that an increase in the brain Na ratio is associated with an increase in EST, it is believed that the relatively acute nature of the electrolyte studies, i.e. the relatively short time interval (4 hours) between the final dose of meprobamate and sacrifice of the animals, did not permit adequate time for a steady state of electrolyte distribution to occur. On the other hand, comparison of the brain Na ratio-EST relation between single-dose treatment and chronic treatment indicates a trend, which suggests that the chronic adminis-

tration of meprobamate tends to diminish the changes in the brain Na ratio at the 1/2- and 4-hour time periods. It is possible that this action of meprobamate on the brain Na ratio may be associated with the development of tolerance to the threshold-raising effect of this drug. The results obtained in the promazine studies are suggestive of a correlation between an increase in the brain Na ratio (see table 4) and withdrawal hypoexcitability, as indicated by an increase in the EST (see E, figure 5). Thus, it is suggested that withdrawal hypoexcitability following chronic promazine treatment may be due, at least in part, to a change in the brain Na ratio.

Woodbury (1958) has demonstrated that the rate of electrolyte exchange from plasma to brain extracellular space is delayed by the blood brain barrier. For this reason, accurate interpretation of the data obtained from the electrolyte studies is difficult. The lag in movement of electrolytes across the blood brain barrier and the acute nature of these studies served to compromise the results. It is believed that studies of this type, which involve a short interval between drug treatment and sacrifice of the animals, can yield unequivocal information only when analytical procedures are designed which are independent of electrolyte equilibrium between plasma and brain extracellular compartment or when methods for calculation are devised to correct for the error introduced by the blood brain barrier.

Since the results of the electrolyte studies in the meprobamate-treated animals is apparently contrary to the accepted concept of correlation between brain Na ratio and brain excitability, it might prove advantageous to employ a longer acting agent in this type of study. Hence, phenaglycodol, a substituted diol with a longer action than meprobamate, or a long-acting barbiturate may be more useful for the study of the mechanism of physical dependence. Since Woodbury and Esplin (1959) have reported a correlation between brain amino acid metabolism and brain excitability, future investigations on mechanism of physical dependence should include the role of brain amino acids, for example, gamma-aminobutyric acid and its congeners, in this phenomenon.

The practical application of the technics employed in these studies should not be overlooked. The data presented indicate that both the electroshock and the chemoshock technics may prove useful for the detection of tolerance and withdrawal hyperexcitability induced by the administration of large doses of drugs which depress the central nervous system. On the other hand, the same technics may prove useful for the detection of withdrawal hypoexcitability induced by chronic administration of large doses of drugs which stimulate the central nervous system.

In view of the fewer animals required without sacrifice of precision and the similar profile of actions obtained from meprobamate, phenaglycodol, and promazine by the 2 procedures (intravenous pentylenetetrazol and low-frequency electroshock), the graded-response technic reported herein should lend itself to the routine screening of candidate drugs for physical dependence liability. It must be emphasized, however, that the predictive value of this test can be determined only after more laboratory and clinical results have been accumulated.

VII. SUMMARY AND CONCLUSIONS

Low-frequency electroshock seizure threshold (EST) and pentylenetetrazol seizure threshold (PST) were employed to measure the effects of chronic administration of meprobamate (Miltown, Equanil), phenaglycodol (Ultran), and promazine (Sparine) and of their subsequent withdrawal on the central nervous system in mice. To provide a basis of reference, the effects of single doses of these same agents on brain excitability, as measured by the EST and PST tests, were also estimated. The EST was determined by means of a Grass stimulator (model S4B). The stimulus parameters employed consist of unidirectional pulses of 0.2 millisecond duration delivered for 3 seconds, through corneal electrodes, at a frequency of 6 pulses per second. The PST was determined by the intravenous infusion of a 0.5% solution of pentylenetetrazol at a rate of 0.005 ml per second until each mouse exhibits persistent clonus for 3 seconds. The animals were chronically treated for 2 to 3 weeks; drug administration was abruptly terminated and the EST and PST were measured at predetermined periods. The results obtained were expressed as threshold ratios (threshold drug group/ threshold control group).

The acute administration of a single 300 mg/kg dose of meprobamate increased the EST-ratio more than 10 fold and the PST-ratio 4.5 fold 1/2 hour after drug treatment; whereas, after approximately 2 weeks of chronic treatment with meprobamate, this same dose of drug increased the EST-ratio only 2.5 fold and the PST-

ratio only 2.9 fold. Thus, tolerance develops to the threshold-raising effects of meprobamate. Four hours after the final dose of meprobamate in the chronically-treated animals, the EST-ratio was somewhat less than 0.8 and the PST-ratio was 0.82. Since the EST and PST 4 hours after the administration of a 300 mg/kg dose of meprobamate to non-tolerant animals were the same as the thresholds of control animals, the reduction in the seizure thresholds observed in the chronically-treated animals must be attributed to the abrupt withdrawal of meprobamate.

The chronic administration of phenaglycodol and its abrupt withdrawal produced responses which essentially paralleled those produced by meprobamate. Acute administration of a single 300 mg/kg dose of phenaglycodol increased the EST-ratio 3.7 fold and the PST-ratio 7.4 fold 1 hour after drug treatment; whereas, after approximately 2 weeks of chronic treatment with phenaglycodol, this same dose of drug increased the EST-ratio only 1.9 fold and the PST-ratio only 4.7 fold. Thus, tolerance develops to the threshold-raising effects of phenaglycodol. After the final dose of phenaglycodol in the chronically-treated animals, the EST-ratio at 8 hours was 0.81 and the PST-ratio at 12 hours was 0.82. Since the thresholds after the administration of a single dose of 300 mg/kg of phenaglycodol to non-tolerant animals were 2.2 fold at 8 hours for the EST-ratio and normal at 12 hours for the PST-ratio, the reduction in seizure thresholds observed in the chronically-treated animals must be attributed to the abrupt withdrawal of phenaglycodol.

The acute administration of a single 37.5 mg/kg dose of promazine to mice decreased the EST-ratio to 0.77 at 4 hours and decreased the PST-ratio to 0.85 at 1 hour. After approximately 2 weeks of chronic treatment with promazine, the EST-ratio was 0.81 at 4 hours and the PST-ratio was 0.76 at 1 hour after promazine treatment. Since the EST- and PST-ratios of the acutely- and chronically-treated animals do not differ, the results suggest that tolerance either does not develop to the threshold-lowering effects of promazine or can not be detected by the technics employed. After the final dose of promazine in the chronically-treated mice, the EST-ratio was 1.18 at 48 hours and the PST-ratio was 1.21 at 72 hours after the final dose of promazine. Since the EST-ratio and the PST-ratio were not elevated at 48 and 72 hours, respectively, after an acute dose of promazine, the increase in the seizure thresholds in the chronically-treated animals must be attributed to the abrupt withdrawal of promazine.

Thus, the EST and PST studies indicate that the chronic administration of large doses of meprobamate or phenaglycodol result in the development of tolerance and physical dependence. Tolerance is manifested by a reduction in the seizure threshold-raising effects of these 2 agents. Physical dependence is manifested by withdrawal hyperexcitability, as indicated by a decrease in EST and PST. The chronic administration of relatively large doses of promazine does not induce tolerance development, but produces physical dependence. Physical dependence to promazine is manifested by withdrawal hypo-

excitability as indicated by an increase in EST and PST. On the basis of clinical reports on central nervous system depressant and excitant drugs and on the basis of the results obtained in the present studies, it is suggested that agents which tend to depress the central nervous system when administered tend to induce withdrawal symptoms characterized by hyperexcitability, whereas, agents which tend to stimulate the central nervous system when administered tend to induce withdrawal symptoms characterized by hypoexcitability.

Since a decrease in the brain Na ratio (extracellular brain Na/intracellular brain Na) can be correlated with an increase in brain excitability and an increase in the brain Na ratio can be correlated with a decrease in brain excitability, it was thought important to investigate the effects of acute and chronic administration and abrupt withdrawal of meprobamate and promazine on the distribution of brain electrolyte in mice. It was believed that such a study may contribute to an understanding of the mechanism of physical dependence. The results of the electrolyte studies in acutely-treated animals indicate that the brain Na ratio tended to increase 1/2 hour after meprobamate and became significantly increased ($P<.05$) at 4 hours. In the chronically-treated animals, the brain Na ratio tended to be increased 4 hours after the final dose of meprobamate, at a time interval when brain excitability was increased, according to the EST determination. This seemingly anomalous response is probably due to a lag period in the exchange of electrolytes between plasma and the

extracellular compartment due to the blood-brain barrier. That is, the animals were analyzed before a steady state of electrolyte distribution occurred and the increased brain Na ratio observed at a time when the EST was either normal or decreased is probably a reflection of the previous interval when brain function was depressed. In the comparison of the acutely- and the chronically-treated animals, a trend was observed in which the brain Na ratio in the latter animals at the 1/2- and 4-hour time periods was diminished. Hence, it is tempting to speculate that an alteration in the brain Na ratio may be associated with the development of tolerance to the seizure threshold-raising effect of meprobamate. In the case of the animals chronically treated with promazine, the brain Na ratio tended to increase 48 hours after the final dose of drug. This observation, although not statistically significant, correlates well with withdrawal hypoexcitability as demonstrated by an increase in the EST at this time period. It is suggested that withdrawal hypoexcitability in the promazine-treated animals may be due, at least in part, to the increase in brain Na ratio.

Because of the complication engendered by the blood-brain barrier in electrolyte studies, especially those of an acute nature as described herein, future studies on the mechanism of physical dependence should employ agents which possess a longer time of action than meprobamate, such as phenaglycodol or long-acting barbiturates. Also, in view of the reported role of amino

acid metabolism in brain function, future studies of this type should include the investigation of brain amino acid metabolism, such as gamma-aminobutyric acid and its congeners.

VIII. APPENDICES

A. Technical Procedures

1. Procedure for obtaining blood

Approximately 10 minutes prior to exsanguination, each mouse is anesthetized by the intraperitoneal injection of 70 mg/kg of pentobarbital sodium (1.4% aqueous solution containing 10% ethanol). A 22 gauge, 3/4- or 1-inch needle is placed on a 2-ml syringe and the plunger and barrel of the syringe are sparingly moistened with a heparin solution. Blood is drawn from the descending abdominal aorta of each mouse, and the blood from 7 to 10 mice is pooled in a test tube to form a single sample. The whole-blood sample is centrifuged and the plasma decanted.

2. Determination of plasma chloride (Cotlove, Thrantham, and Bowman, 1958)

a. Procedure

Preceding a series of analyses, the electrodes of the automatic chloride titrator (Cotlove Chloridometer, Laboratory Glass and Instruments Corp.) are first conditioned by the titration of a chloride standard; the first value obtained is usually low and is not recorded. Two blanks (4 ml of nitric-acetic acid reagent) and 2 standards (0.1 ml of 160 mEq chloride per liter with 4 ml of acid reagent) are titrated at the beginning of the determinations and 1 of each after every

10 to 20 samples. The plasma samples are prepared by adding 4 ml of the nitric-acetic acid reagent to 0.1 ml of plasma in a glass titration vial. At the time of the titration, whether blank, standard, or plasma sample, 10 drops of gelatin reagent (approximately 0.5 ml) are added to the glass vial, which is then placed in position on the chloride titrator. The rate switch is set on high and the titration switch is turned to position no. 1. When the indicator (black) pointer becomes stabilized, the adjustable (red) pointer is set 10 microamperes above the indicator (black) pointer. The timer is then set to zero and titration is begun by turning the titration switch to position no. 2. When the analysis is completed, the timer is automatically stopped; the elapsed time is recorded. The vial is removed from the titrator, the electrodes are rinsed with chloride-free water and the procedure is repeated with each subsequent glass vial.

b. Calculation of plasma Cl concentration

(1) Gross seconds = timer reading

(2) Average net seconds of standards = (average gross seconds of standards)-(average gross seconds of blanks) = 62.09-0.97 = 61.12

(3) Net seconds of plasma sample = (gross seconds of plasma sample)-(average gross seconds of blanks) = 46.85-0.97 = 45.88

(4) Factor = K =

$$\frac{\text{(ml of standard) x (concentration of standard)}}{\text{average net seconds of standards}} = \frac{0.1 \times 160}{61.12} = 0.2618$$

(5) Concentration of Cl, mEq/l plasma =

$$K \times \frac{\text{(net seconds of plasma sample)}}{\text{(ml of plasma sample)}} = \frac{0.261 \times 45.88}{0.1} = 120.11$$

c. Reagents

(1) One-tenth normal nitric acid-10% acetic acid. One-hundred ml of reagent grade glacial acetic acid and 6.4 ml of reagent grade concentrated nitric acid are added to 900 ml of water.

(2) Gelatin reagent. (a) Gelatin (Knox unflavored gelatin), thymol blue (water soluble) and thymol crystals (reagent grade) were mixed in the proportions of 60:1:1 by weight, respectively. (b) To 6.2 g of the above dry mixtures, 1 liter of hot water is added and the mixture heated gently with continuous swirling until the solution is clear. After cooling, the solution is stored in the refrigerator. (c) For each day's analyses, a new portion of the gelatin reagent is removed from the refrigerator and liquified by immersion in hot water.

(3) Sodium chloride standard, 160 mEq/l. Dried, reagent grade sodium chloride, 9.3520 g, is dissolved in sufficient water to make 1 liter of solution.

3. Determination of plasma concentration of water, potassium, and sodium

a. Procedure for determination of plasma water

A 1 ml aliquot of plasma is weighed in a tared platinum crucible and dried in an oven at 105° C for 24 hours. After cooling, the crucible is again weighed. The difference in weight represent mg of water per ml of plasma. This value multiplied by $\frac{1000}{1000}$ represents g of water per l plasma.

b. Procedure for determination of plasma potassium and sodium

The plasma, dried as described above, is moistened with 4 drops of concentrated sulfuric acid and the crucible is placed in a muffle furnace. The plasma is heated at 650° C for 90 minutes. After the furnace has cooled, the crucible is removed. One ml of concentrated hydrochloric acid is added to the crucible and slowly evaporated on a hot plate. The residue is then dissolved by the addition of 10 ml of a lithium chloride solution (10 mEq/l) to make a 1:10 dilution. A sample of this dilution (1:10) is then tested in the flame photometer (Baird-Atomic, Inc., Model DB4) for the concentration of plasma potassium. In addition, standard solutions containing 0.5 and 0.3 mEq/l of K are tested at the beginning of a series of determinations and after every 3 to 5 samples. A 1 ml aliquot of the 1:10 dilution is then further diluted to 25 ml with the 10 mEq/l lithium chloride solution (final dilution is 1:250). A sample of this dilution is then tested

in the flame photometer for plasma Na concentration. For plasma Na determinations, standard solutions containing 0.7 and 0.5 mEq/l of Na are employed.

c. Calculation of plasma K concentration

(1) Galvanometer reading of sample = 565

(2) Galvanometer reading of high K standard (0.5 mEq/l) = 800

(3) Galvanometer reading of low K standard (0.3 mEq/l) = 480

(4) Difference between concentration of high and low K standards = 0.5 - 0.3 = 0.2

(5) Dilution factor = 10

(6) Specific gravity of plasma = 1.008

(7) mEq/l of K in diluted sample =

$$\frac{[(1) - (3)] \times (4)}{(2) - (3)} + \text{concentration of low standard} =$$

0.3531

(8) Concentration of K, mEq/kg plasma =

$$\frac{(7) \times (5)}{(6)} = \frac{0.3531 \times 10}{1.008} = 3.50$$

d. Calculation of plasma Na concentration

Plasma Na concentration is determined by the same procedure described for plasma K with the following exceptions:

(1) Different concentrations of standard solutions are employed;

(2) A 1:250 dilution of plasma is tested on the flame photometer and the appropriate dilution factor (250) is substituted in the calculations (see above).

e. Plasma Na and K standards

Electrolyte	Concentration of cation in mEq/l		
NaCl	0.7	0.5	0.3
KCl	0.7	0.5	0.3
LiCl	10.0	10.0	10.0

4. Determination of brain tissue water, potassium, and sodium

a. Procedure for brain water

After exsanguination each mouse is decapitated and the cerebral cortex is removed for analysis. The brain tissue of 7 to 10 mice is placed in a tared glass vial and weighed (wet weight). The tissue is then dried at 105° C for 4 days. The tissue is again weighed (dry weight). The concentration of water in brain tissue is calculated as follows:

$$g\ H_2O/kg \text{ wet brain tissue} = \frac{(\text{wet weight} - \text{dry weight}) \times 1000)}{\text{wet weight}}$$

b. Procedure for determination of brain potassium and sodium

The dried brain tissue is triturated, by means of a glass rod, to a powder in the same glass vial in which the sample was dried. Approximately 50 mg of the powdered tissue are introduced into a platinum crucible, moistened with 6 drops of 4N H_2SO_4 and placed in a muffle furnace. The tissue is

heated to 650° C for 24 hours. Two ml of concentrated HCl are then added to the crucible and slowly evaporated on a hot plate. The crucible is allowed to cool and the residue is dissolved in 5 ml of ion-free water. Four ml of the dissolved sample are introduced into a 25-ml volumetric flask and 5 ml of lithium chloride solution (50 mEq/l) are added. The contents of the flask is then diluted to 25 ml with ion-free water and tested on the flame photometer. The concentrations of K and Na are determined in the manner as previously described under plasma K and Na (see page 82 The concentrations of electrolyte in the standard solutions are described under (e.) below.

c. Calculation of brain tissue K concentration

(1) Galvanometer reading of sample = 709

(2) Galvanometer reading of high K standard (0.9 mEq/l) = 804

(3) Galvanometer reading of low K standard (0.7 mEq/l) = 642

(4) Difference between concentration of high and low K standards = 0.9 - 0.7 = 0.2

(5) Dilution factor = 31.25

(6) Weight of brain sample = 0.049 g

(7) Per cent dry weight of tissue =

$$100.00 - \left[\frac{\text{wet weight - dry weight}}{\text{wet weight}} \times 100\right] = 21.35\%$$

(8) Concentration of K, mEq/l =

$$\frac{[(1) - (3)] \times (4)}{(2) - (3)} + \text{concentration of low standard} =$$

$$\frac{(709 - 642) \times 0.2}{804 - 642} + 0.7 = 0.7827$$

(9) Concentration of K, mEq/kg wet brain tissue =

$$\frac{(8) \times (5)}{(6)} \times (7) = \frac{0.7827 \times 31.25}{0.049} \times 0.2135 =$$

106.15

d. Calculation of brain tissue Na concentration

Brain tissue Na concentration is calculated by the same procedure described for brain K except that less concentrated standards are employed (0.2 - 0.5 mEq/l NaCl).

e. Brain Na and K standards

Electrolyte	Concentration of cation in mEq/l				
NaCl	0.9	0.7	0.5	0.3	0.2
KCl	0.9	0.7	0.5	0.3	0.2
LiCl	10.0	10.0	10.0	10.0	10.0

5. Determination of brain tissue chloride (Van Slyke and Sendroy, 1923)

a. Procedure

(1) Alkali digestion of tissue. Approximately 50 mg of dried brain tissue are introduced into a 25-ml Erlenmeyer flask and moistened with 1 ml of 1N KOH solution. The mixture is heated on a water

bath for 3 hours (small amount of ion-free water is added after approximately 1 hour of heating). Two blanks, each consisting of 4 ml of 1N KOH, and 2 standards, each consisting of 4 ml of 1N KOH and 2 ml of a standard NaCl solution (approximately 10 mEq/l), are also heated. After removal from the water bath, the solutions are cooled and neutralized with equivalent volumes of 1N HNO_3. All 3 solutions (blanks, standards, and unknowns) are then assayed for chloride.

(2) Assay for Cl. Three ml of 0.025N $AgNO_3$ solution in concentrated HNO_3 are added to each Erlenmeyer flask. The solutions are then heated an additional 3 hours on a water bath, allowed to cool, and 6 ml of 5% ferric alum added. Finally, each solution is titrated with 0.02N NH_4SCN until a pink color persists for 15 seconds.

b. Calculation of brain Cl concentration (mEq/kg, wet tissue)

Table 7. Values for Calculation of brain chloride concentration

	sample weight (g)	ml 0.02N NH_4SCN	ml NH_4SCN blank - ml NH_4SCN	% dry weight
Unknown	0.0514	3.370	0.359	21.35
Cl standard (average)	----	2.721	1.008	----
Blank (average)	----	3.729	----	----

(1) The concentration of the standard Cl solution is multiplied by 0.002 and the product obtained is divided by the difference between ml NH_4SCN solution used for the blank (3.729) and ml NH_4SCN solution used for the standard (2.721):

$$\frac{10.002 \times 0.002}{1.008} = 0.01985 = F$$

(2) The difference in ml NH_4SCN solution used to titrate the blank (3.729) and the sample (3.370) is then multiplied by F: $0.359 \times 0.01985 = 0.00712615$

(3) Concentration of Cl, mEq/kg wet-brain tissue:

$$\frac{(2)}{\text{sample weight}} \times \% \text{ dry weight} \times 1000 =$$

$$\frac{0.0071265}{0.0514} \times (0.2135 \times 1000) = 29.55$$

6. Method for calculation of intracellular and extracellular space[a]

Symbol	Definition	Method for calculation
$(Na)_p$	mEq Na per kg plasma	as measured
$(K)_p$	mEq K per kg plasma	as measured
$(Cl)_p$	mEq Cl per kg plasma	as measured
$(H_2O)_p$	g H_2O per kg plasma	as measured

[a]The subscript p refers to plasma, b to moist cerebral cortex (brain), e to extracellular, i to intracellular, and c to intracellular water.

$(Na)_e$	mEq Na per kg extracellular H_2O	$\frac{(Na)_p \times (0.95) \times (1000)}{(H_2O)_p}$
$(K)_e$	mEq K per kg extracellular H_2O	$\frac{(K)_p \times (0.95) \times (1000)}{(H_2O)_p}$
$(Cl)_e$	mEq Cl per kg H_2O in plasma	$\frac{(Cl)_p \times (1000)}{(H_2O)_p \times (0.95)}$
$(Na)_b$	mEq Na per kg moist cerebral cortex	as measured
$(K)_b$	mEq K per kg moist cerebral cortex	as measured
$(Cl)_b$	mEq Cl per kg moist cerebral cortex	as measured
$(H_2O)_b$	g H_2O per kg moist cerebral cortex	as measured
$(H_2O)_e$	Cl space (extra-cellular space)	$\frac{(Cl)_b \times (1000)}{(Cl)_e}$
$(H_2O)_c$	Cl-free space intra-cellular space)	$(H_2O)_b - (H_2O)_e$
$(Na)_{eb}$	mEq Na per kg moist brain, extracellular	$\frac{(Na)_e \times (H_2O)_e}{(1000)}$
$(Na)_{ib}$	mEq Na per kg moist brain, intracellular	$(Na)_b - (Na)_{eb}$
$(K)_{eb}$	mEq K per kg moist brain, extracellular	$\frac{(K)_e \times (H_2O)_e}{(1000)}$
$(K)_{ib}$	mEq K per kg moist brain, intracellular	$(K)_b - (K)_{eb}$

$(Na)_c$	mEq Na per kg intracellular H_2O	$\frac{(Na)_{ib} \times (1000)}{(H_2O)_c}$
$(K)_c$	mEq K per kg intracellular H_2O	$\frac{(K)_{ib} \times (1000)}{(H_2O)_c}$
	brain Na ratio	$\frac{(Na)_e}{(Na)_c}$
	brain K ratio	$\frac{(K)_c}{(K)_e}$

B. Statistical Procedures

1. Graphic log-probit method for evaluating EST experiments (Litchfield and Wilcoxon, 1949)

a. Determination of the CC50 and the fCC50

The following is an example of the method employed for the determination of the CC50 and the fCC50. The data shown in table 8 are taken from a typical experiment (EST 60 minutes after phenaglycodol, 200 mg/kg) as described in the General Procedure.

Table 8.

Values for calculation of $(Chi)^2$

Volts	Number Animals Tested	Number Seizures	Observed % Seizures	Expected % Seizures	Observed Minus Expected (Absolute Value)	Contributions to $(Chi)^2$ (Obtained from Nomograph 1)
40	10	3	30	29	1	0.001
45	12	6	50	50	0	0.000
50	10	7	70	69	1	0.001
55	10	8	80	82.5	2.5	0.003

The % of animals that exhibited seizures (observed %) is plotted by the dots in figure 7. A straight line (regression line is then visually fitted to the points. Expected % of seizures is determined by projecting a vertical line from the observed % point to the regression line. The point at which the vertical line intersects the regression line is the

expected %. For example, the expected % of positive responses following stimulation with 55 volts is 82.5% (as shown by "x" in figure 7). The absolute difference between each pair of observed and expected % values is obtained and its contribution to $(Chi)^2$ determined from nomograph No. 1 in the original paper by Litchfield and Wilcoxon (1949). The values thus obtained constitute the figures in the right-hand column of table 8. The contributions to $(Chi)^2$ are totaled and the sum (0.005) is multiplied by the average number of animals per dose (12.5). This is the $(Chi)^2$ of the line. If the $(Chi)^2$ of the line is less than the value of $(Chi)^2$ taken from table II of the original paper for 2 degrees of freedom (number of volts plotted minus 2 = 2) the data are not significantly heterogeneous, i.e. the line is a good fit. In the present example. the product of 0.005 x 12.5 is less than 5.99 (from table II of original paper), therefore, the data are not significantly heterogenous. The CC16, CC50, and CC84 are obtained from the dose (volt)-response curve, and the fCC50 is calculated as follows:

$$\text{Slope function} = \frac{CC84/CC50 + CC50/CC16}{2} =$$

$$\frac{55.5/45.0 + 45.0/36.5}{2} = 1.23$$

$fCC50 = 1.23^{(2.77/\sqrt{N})}$, where N is the number of animals whose expected effects are between 16 and 84%

$$= 1.23^{(2.77/\sqrt{42})} = 1.23^{(0.43)} = 1.10$$

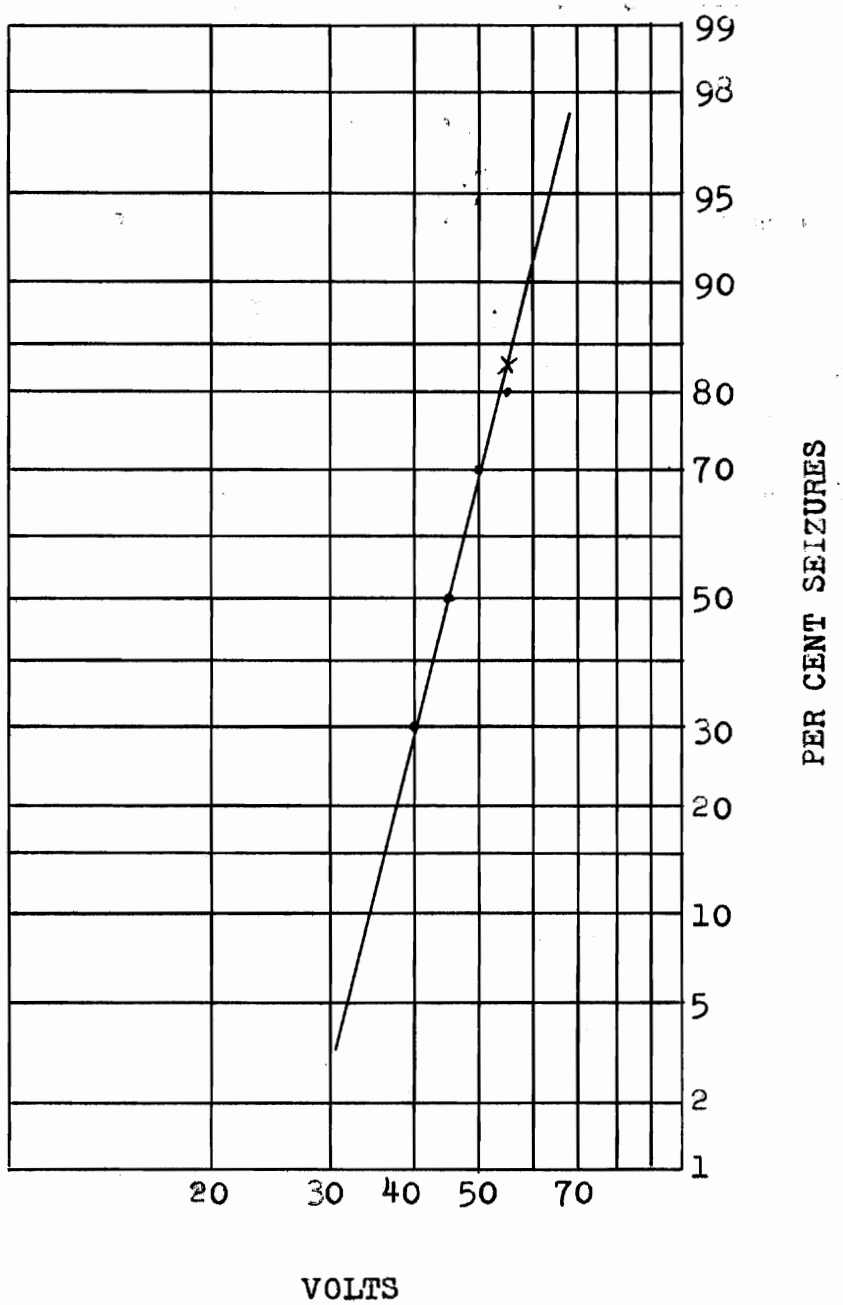

Figure 7. Per cent of animals which exhibited seizures plotted on log-probability paper.

b. Calculation of EST ratio (threshold drug group/threshold control group)

(1) CC50 of control group = 17.4 volts

(2) fCC50 of control group = 1.08

(3) CC50 of drug treated group = 45.0 volts

(4) fCC50 of drug treated group = 1.10

(5) EST ratio = CC50 drug group/CC50 control group = 45.0/17.4 = 2.59

(6) fEST ratio = 1.16 (obtained from nomograph No. 4 of original paper) with the use of (2) and (3) above

(7) The 95% fiducial limits for the EST ratio are obtained by first dividing and then by multiplying the ratio by the fEST as follows:

Lower limit = EST ratio/fEST ratio =

2.59/1.16 = 2.23

Upper limit = EST ratio x fEST ratio =

2.59 x 1.16 = 3.00

Thus, the EST ratio and 95% fiducial limits =

2.59 (2.23 -- 3.00)

2. Analysis of covariance method for evaluating PST experiments in mice

The following is an example of the method employed for the calculation of the PST ratio (threshold drug group/threshold control group). The data employed are taken from a typical experiment (PST 60 minutes after promazine, 37.5 mg/kg) as described in the General Procedure. The weights of the mice and the experimental observations, as well as the logarithms of these values, are

summarized in section a. below. These values are used to calculate the sums, sums of squares, sums of products, and corrections for means of groups, which are summarized in section b. below. From the values shown in section b., the analysis of variance is completed in section c. below and the PST ratio and its 95% fiducial limits are calculated in section d. below. Additional details of the calculation may be found in Finney (1952), pp. 43-56.

a. Weights, experimental observations, and logarithms

n	Body Weight (g)	X (log g)	Infusion Time (sec)	Y (log sec)
Group I: H_2O (control)				
1	30.0	1.4771	52.5	1.7202
2	27.0	1.4314	35.0	1.5441
3	28.5	1.4548	35.0	1.5441
4	29.5	1.4698	43.0	1.6335
5	30.0	1.4771	53.0	1.7243
6	27.0	1.4314	50.5	1.7033
7	29.0	1.4624	38.0	1.5798
8	29.0	1.4624	39.0	1.5911
9	28.5	1.4548	35.0	1.5441
10	26.5	1.4233	46.5	1.6675
Group II: Promazine, 1 hour after 37.5 mg/kg				
1	28.0	1.4472	29.0	1.4624
2	25.0	1.3979	27.5	1.4393
3	29.0	1.4624	40.0	1.6021
4	29.0	1.4624	41.0	1.6128
5	30.5	1.4843	39.5	1.5966
6	28.0	1.4472	32.0	1.5052
7	27.0	1.4314	34.5	1.5378
8	30.5	1.4843	39.0	1.5911
9	28.5	1.4548	36.0	1.5563
10	27.5	1.4393	38.5	1.5855

b. Sums, sums of squares, sums of products and corrections for means

Sums, sums of squares, and sums of products

	ΣX	ΣX^2	$\Sigma(XY)$	ΣY	ΣY^2	n
Group I	14.5445	21.1577	23.6403	16.2520	26.4625	10
Group II	14.5112	21.0634	22.4870	15.4891	24.0251	10
Sum	29.0557	42.2211	46.1273	31.7411	50.4876	20

Corrections for means

	$(\Sigma X)^2/n$	$(\Sigma X)(\Sigma Y)/n$	$(\Sigma Y)^2/n$
TOTAL	42.2117	46.1130	50.3749
Group I	21.1542	23.6377	26.4128
Group II	21.0575	22.4765	23.9912
Sum	42.2117	46.1142	50.4040

c. Analysis of variance

Source of Variation	Sums of squares and products				Adjusted			f	P
	d.f.	$\leq(X-\bar{X})^2$	$\leq(X-\bar{X})(Y-\bar{Y})$	$\leq(Y-\bar{Y})^2$	d.f.	$\leq(Y-\bar{Y})^2$	Mean Square		
Between groups	1	.0000	.0012	.0291	1	.0258	.0258	6.789	<.05
Within groups	18	.0094	.0131	.0836					
Regression					1	.0182	.0182	4.789	<.05
Error					17	.0654	.0038		
TOTAL	19	.0094	.0143	.1127					

b = regression coefficient = .0131/.0094 = 1.3936

Adjusted $\leq(Y-\bar{Y})^2 = b^2\leq(X-\bar{X})^2 - 2b\leq(X-\bar{X})(Y-\bar{Y}) + \leq(Y-\bar{Y})^2$

d. Summary of effect of promazine on PST

Adjusted means

Group	$\bar{X}$	$\bar{X}-\bar{\bar{X}}$	$b(\bar{X}-\bar{\bar{X}})$	$\bar{Y}$	$\bar{Y}$corr
I	1.4545	.0017	.0024	1.6252	1.6228
II	1.4511	-.0017	-.0024	1.5489	1.5513

$\bar{\bar{X}} = (\bar{X}_I + \bar{X}_{II})/2 = 1.4528$

Threshold ratio

M = log threshold ratio = $\bar{Y}_{II}$corr - $\bar{Y}_I$corr = 1.5513 - 1.6228 = $\bar{1}$.9285

R = threshold ratio = antilog of M = 0.848

95% Fiducial limits

f = 1 + .0000/.0094 = 1.0000

$V(\bar{Y}_I corr)$ = adjusted mean square for error/n_I x f = .0038

$V(\bar{Y}_{II} corr)$ = adjusted mean square for error/n_{II} x f = .0038

$V(M) = V(\bar{Y}_I corr) + V(\bar{Y}_{II} corr) = .0076$

$S_M = \sqrt{V(M)} = .0276$

95% fiducial limits for M = $M \pm (t_{.05} \times S_M)$ =

$\bar{1}.9285 \pm (2.011 \times .0276)$ =

$\bar{1}.8730$ -- $\bar{1}.9840$

95% fiducial limits for R = antilog 95% fiducial limits for M =

0.747 -- 0.964

Therefore, the PST ratio and 95% fiducial limits =

0.85 (0.75 -- 0.96)

3. Procedure for calculation of mean, standard deviation of mean, and "t" test for significance

a. Definition of terms

x = individual observation

n = number of observations

S = sum of

$\bar{x} = S(x)/n$ = mean of observations

$S(x-\bar{x})^2$ = sums of squares of deviation about mean

$S.D._{\bar{x}} = \sqrt{S(x-\bar{x})^2/n(n-1)}$ = standard deviation of the mean

b. Calculation of mean plus or minus standard deviation of mean for 2 groups of observations

	Plasma K (mEq/kg)	
	Group A	Group B
	3.46	3.90
	3.71	3.81
	3.58	3.50
	3.98	3.39
Sx	14.73	14.60
n	4	4
$\bar{x}$	3.68	3.65
Sx^2	54.39	53.47
$(Sx)^2/n$	54.24	53.29
$S(x-\bar{x})^2$	0.15	0.18
$n(n-1)$	12	12
$S(x-\bar{x})^2/n(n-1)$	0.012	0.015
$S.D._{\bar{x}}$	0.11	0.12
$\bar{x} \pm S.D._{\bar{x}}$	3.68 ± 0.11	3.65 ± 0.12

c. "t" test for significant difference between means

$$t = \frac{\bar{x}_A - \bar{x}_B}{\sqrt{\frac{(1/n_A + 1/n_B)\left[S(x_A - \bar{x}_A)^2 + S(x_B - \bar{x}_B)^2\right]}{(n_A - 1) + (n_B - 1)}}}$$

$$t = \frac{3.68 - 3.65}{\sqrt{\frac{(1/4 + 1/4)(0.15 - 0.18)}{(3 + 3)}}}$$

$t = 0.03/0.17 = 0.002$

The t value calculated above is compared with a value obtained from a "t" table. The table is entered with 6 degrees of freedom $(n_A - 1) + (n_B - 1)$ and the value 2.45 (P of .05) is obtained. Since 0.002 is less than 2.45, the probability that the 2 means are not significantly different is greater than .05 ($P > .05$).

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EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF PSYCHOPHARMACOLOGIC DRUGS ON EXPERIMENTAL SEIZURE THRESHOLD

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Low-frequency electroshock seizure threshold (EST) and pentylenetetrazol (Metrazol) seizure threshold (PST) were employed to measure the effects of chronic administration of meprobamate (Miltown, Equanil), phenaglycodol (Ultran), and promazine (Sparine) and of their subsequent withdrawal on the sensitivity of the central nervous system in mice. To provide a basis of reference, the effects of single doses of these same agents on brain excitability, as measured by the EST and PST tests, were also estimated. The EST was determined by means of a Grass stimulator (model S4B). The stimulus parameters employed consist of unidirectional pulses of 0.2 millisecond duration delivered for 3 seconds, through corneal electrodes, at a frequency of 6 pulses per second. The PST was determined by the intravenous infusion of a 0.5% solution of pentylenetetrazol at a rate of 0.005 ml per second until each mouse exhibited persistent clonus for 3 seconds. The animals were chronically-treated for 2 to 3 weeks; drug administration was abruptly terminated and the EST and PST were measured at predetermined periods. The results obtained were expressed as threshold ratios (threshold drug group/threshold control group).

The results of the electroshock and chemoshock studies indicate that both meprobamate and phenaglycodol increased EST and PST. Chronic administration of either drug for approximately 2 weeks decreased the EST- and PST-raising effects of both drugs, i.e. resulted in the development of tolerance. When chronic

administration of either agent was abruptly terminated the EST and PST were decreased; this response was interpreted as withdrawal hyperexcitability or physical dependence. In contrast, promazine decreased the EST and PST. Chronic administration of this drug over a period of approximately 3 weeks had no significant effect on the EST- or PST-lowering effect. This observation indicates that tolerance to the threshold-lowering effect of promazine either did not occur or could not be measured by the experimental technics employed. Upon abrupt termination of promazine administration, the EST and PST were increased; this response was interpreted as withdrawal hypoexcitability or physical dependence.

The effects of a single dose of meprobamate or promazine and of the chronic administration of these same agents on electrolyte and water concentration in plasma and cerebral tissue of mice were studied in an effort to reveal changes which might explain, at least in part, the phenomena of tolerance and physical dependence. The results of the electrolyte studies indicate that there is no consistent correlation between brain electrolyte alterations and brain excitability. Indeed, some of the alterations in brain Na ratio were seemingly contradictory to the accepted concept that an increased Na ratio is associated with an increased EST. However, it was quite likely that the acute nature of the electrolyte studies, i.e. the short interval between drug treatment and sacrifice of the animals for analyses, may have compromised the results. Since the blood brain barrier retards the rate of electro-

lyte movement from plasma into the brain extracellular compartment, it was obvious that the electrolyte studies reported were done while brain water and electrolytes were in an unsteady state.

The data presented indicate that both the electroshock and the chemoshock technics may prove useful for the determination of tolerance and withdrawal hyperexcitability induced by the administration of large doses of drugs which depresses the central nervous system. On the other hand, these same technics may prove useful for the detection of withdrawal hypoexcitability induced by chronic administration of large doses of drugs which stimulates the central nervous system.